(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 May 2005 (06.05.2005)

PCT

(10) International Publication Number
WO 2005/039631 A1

(51) International Patent Classification[7]: A61K 39/00, A61P 35/00, 31/00, A61K 39/39

(21) International Application Number: PCT/EP2004/011950

(22) International Filing Date: 11 October 2004 (11.10.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0323840.9 10 October 2003 (10.10.2003) GB

(71) Applicant *(for all designated States except US)*: **ISTITUTO SUPERIORE DI SANITA** [IT/IT]; Viale Regina Elena 299, I-00161 Rome (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CAPUTO, Antonella** [IT/IT]; Via Nazionale 33, San Nicolo (Ferrara), I-44040 (IT). **GAVIOLI, Riccardo** [IT/IT]; Via Carlo Mayr 137, I- 44100 Ferrara (IT). **ENSOLI, Barbara** [IT/IT]; Via Monte Pollino, 2, I- Rome (IT).

(74) Agent: **LORD, Hilton, David**; Marks & Clerk, 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: VACCINES CONTAINING THE HIV TAT PROTEIN AS AN ADJUVANT FOR THE ENHANCEMENT OF CYTOTOXIC T-CELL RESPONSES

(57) Abstract: Tat, when used in a vaccine, causes MHC-I to expose subdominant epitopes present within an antigen, thereby enabling an optimal immune response to be generated, within an individual, against the antigen and variants of the antigen, such as might be encountered with HIV or influenza viruses.

WO 2005/039631 A1

VACCINES CONTAINING THE HIV TAT PROTEIN AS AN ADJUVANT FOR THE ENHANCEMENT OF CYTOTOXIC T-CELL RESPONSES

FIELD OF THE INVENTION

The present invention relates to vaccines comprising Tat, biologically active derivatives thereof or precursors therefor, including nucleic acids encoding such, as well as to methods for vaccination comprising the use of such vaccines.

BACKGROUND OF THE INVENTION

The Tat protein of HIV-1 is produced very early upon virus entry and is required for virus replication and infectivity. Recently, we have shown that biologically active Tat is very efficiently taken up by dendritic cells and activates them, increasing Th-1 type responses against heterologous antigens. In addition, Tat-based vaccines in monkeys have been shown to be safe, and to induce protective immunity which correlates with the generation of Th-1 type immune responses.

Tat is a regulatory protein of HIV-1 and is produced very early after infection. It is essential for HIV-1 gene expression, replication, and infectivity. During acute infection of T cells by HIV-1, Tat is released in the extracellular milieu in a biologically active form in the absence of cell death or permeability changes[1,2]. Extracellular Tat is taken-up by neighbour cells where it modulates cellular functions, depending on the concentration, oxidation state, and cell type.

In EP-A-1279404, we show that biologically active monomeric Tat protein is very efficiently taken-up by monocyte-derived dendritic cells (DC) and that, after internalisation, it induces DC maturation and augments allogeneic and antigen-specific presentation by DC, increasing Th-1 responses against recall antigens[3]. Fanales-Belasio *et al.* (Journal of Immunology 2002, vol 168 (1), pp. 197-206) also discloses the ability of Tat to augment presentation.

In addition, studies in mice and monkeys have shown that Tat-based vaccines are safe and induce protective immunity against pathogenic virus challenge that correlates with Th-1 type immune responses and cytotoxic T cells[4,5].

Cytotoxic lymphocytes (CTLs) play an essential role in the control of intracellular pathogens, including HIV, suggesting that vaccines eliciting optimal CTL responses have applications for the prevention and/or for the control of virus-associated diseases and tumours.

CTLs recognise peptide epitopes expressed at the surface of target cells in association with MHC class I molecules[6]. The epitope is generated in the cytosol by degradation of the antigen, from where it is transported into the endoplasmic reticulum, where it associates with newly synthesised class I molecules. Often, CTL responses are directed to a single immunodominant peptide out of a larger number of potential epitopes within the same antigen. This phenomenon, known as immunodominance, is still poorly understood. However, the generation and presentation of peptides, the availability of responsive T cells, and little understood immunoregulatory effects can all influence the activation of an efficient immune response to a particular epitope.

The major enzymatic activity responsible for the generation of class I-associated peptides is the proteasome, a large multicatalytic protease that is essential for the degradation of intracellular proteins and the maintenance of cell viability[7,8].

Proteasomes consist of a 20S catalytic core arranged as four heptameric rings. The two outer rings contain structural α-subunits (α1-α7), while the inner rings contain β subunits (β1-β7), three of which (β1, β2, β5) exert catalytic activity through a nucleophilic attack on the peptide bond by the N-terminal threonine[9]. Biochemical studies on the specificities of the proteasome reveal three distinct proteolytic components, which are involved in chymotryptic, tryptic and post-acidic (also called caspase-like) hydrolysing activities. Analysis of the contribution of the individual β-subunits has demonstrated a clear correlation between the individual subunits and the cleavage after preferred amino acids[10]. When cells are exposed to IFN-γ, the three catalytic β-subunits are substituted by LMP2, LMP7 and MECL1 (also referred as

LMP10). These subunits are also expressed in a constitutive manner in specific cell types such as dendritic cells and B cells[11,12], and their incorporation in the proteasome alters its activity and enhances the production of certain peptides[13].

Proteasomes equipped with LMP2, LMP7 and MECL1 have been called immunoproteasomes, as distinct from the constitutively expressed standard proteasomes. The catalytic activity of immunoproteasomes is characterised by a reduced cleavage after acidic amino acids and an increased cleavage after hydrophobic and basic residues, the most frequent residues found at the COOH terminus of MHC class I binding peptides[14]. It has been demonstrated that proteasomes generate the exact COOH terminus of MHC class I binding peptides, whereas the NH_2-terminal cleavage is not always as precise and that aminopeptidases located in the endoplasmic reticulum may cut the NH_2 extensions to generate the correct peptide epitope[15-18].

For full and regulated proteasome function, the 20S proteasome core must assemble with other proteasome components, such as the 19S cap complex, to form the 26S proteasome which is able to degrade ubiquitin-conjugated proteins or/and the PA28 proteasome regulator to form the PA28-proteasome complex. The association of PA28 with the 20S proteasome seems to favour the generation of immunogenic peptides[19]. The generation of immunogenic peptides is a critical step in the activation of epitope-specific CTL responses. Indeed, there is evidence demonstrating that proteasome-mediated proteolysis contributes to the hierarchy of epitopes presented by MHC class I molecules. Subdominant T cell epitopes, in contrast to the immunodominant epitopes, are generated with less efficiency or are destroyed at cleavage sites located within the epitope[20,21].

Cafaro *et al* (Nature Medicine (1999), Vol 5, pp. 643-650), shows that the use of biologically active Tat in an HIV-1 vaccine for monkeys is safe and elicits a broad (both cellular and humoral) but specific immune response and reduces infection with SIV.

WO 00/43037 discloses that Tat and Nef are chemotactic agents for CD4+ cells and that vaccine efficacy may be boosted by the recruitment of CD4+ cells to the site of vaccine injection, when said vaccine is supplemented with Tat and Nef.

WO 02/019968 discloses a co-expression DNA vaccine (CED) that displays immunogenic properties. In particular, a vaccine encoding both an antigen and Tat is disclosed, the antigen benefiting from Tat-mediated immune deviation or immunomodulation/immunoregulation.

We have now, surprisingly, found that the Tat protein induces modifications of the subunit composition of immunoproteasomes in cells either expressing Tat or exposed to exogenous, biologically active Tat protein. In particular, Tat up-regulates the expression of the IFN-γ inducible catalytic subunits LMP7 and MECL1, but down-modulates LMP2. These changes correlate with an increase of all three of the major proteolytic activities of the proteasome. Proteasomes play a key role in the production of MHC class I binding peptides, and we found that Tat decreases the generation and presentation of immunodominant epitopes, while increasing the generation and presentation of subdominant T cell epitopes.

We have also found that modulation of proteosome subunit composition may be achieved by not only wild type Tat, but also by mutated Tat and Tat-derived peptides.

SUMMARY OF THE INVENTION

Thus, in a first aspect, the present invention provides the use of Tat, a biologically active equivalent, or a precursor therefor, in the preparation of a vaccine suitable to elicit an immune response against an antigenic substance having a plurality of epitopes, the epitopes including both immunodominant and sub-dominant epitopes, the vaccine comprising at least a part of the antigenic substance encoding or comprising a sub-dominant epitope thereof.

Thus, Tat, when used in a vaccine, causes MHC-I to expose subdominant epitopes of a variable antigen, thereby enabling a persistent immune response to be generated

within an individual against variants of the antigen, such as might be encountered with HIV or influenza viruses.

In a preferred embodiment, there is provided the use of Tat, a biologically active equivalent thereof or a precursor therefor, in the preparation of a vaccine suitable to elicit an immune response against a plurality of strains of an infectious organism, the vaccine comprising antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope.

It will be understood that a "precursor" includes any suitable material leading to the presence of Tat in the patient in a manner suitable to act as an adjuvant. This may include peptide precursors, such as fusion proteins, including fusions with signal peptides, which may be cleaved to yield active Tat, or which may be active without cleavage, or may include nucleic acid sequences in a form suitable to be expressed *in situ*.

Preferably, the Tat used is the wild type Tat shown in SEQ ID NO 284 or is a mutant and/or fragment thereof.

In one aspect, Tat is mutated. Any number of mutations, whether by substitution, deletion or insertion is envisaged, provided that the mutant is capable of increasing the number of subdominant epitopes presented, preferably by modulation of the proteosome subunits, as described above.

Preferably, the mutant has 90% homology to wild type Tat, according to SEQ ID NO 284, preferably 95% and more preferably 99% homology or sequence identity, as measured by known methods, such as the BLAST program.

In a particularly preferred embodiment, Tat is mutated at position 22. Preferably, the cysteine residue present in the wild type Tat at this position is substituted, preferably by glycine. Other suitable amino acids may also be used, such as alanine or any other non-polar amino acid.

In one embodiment, it is preferred that a fragment of Tat is used in the present invention. Any length peptide may be employed, provided that the above effect is seen. It is particularly preferred, however, that the fragment comprises or encodes at least amino acid numbers 47-86 of SEQ ID NO 284, which are given separately as SEQ ID NO 285. Preferably, the precursor is a polynucleotide, preferably DNA or RNA, encoding at least the above amino acids. It is also preferred that the fragment is a polypeptide comprising these amino acids. More preferably, the polypeptide consists of amino acids 47-86 of SEQ ID NO 284.

Preferably, however, the fragment comprises or encodes for up to: 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 or 200 or more amino acids.

Preferably, Tat may be expressed *in situ*, preferably in a target cell. The cell may be targeted, preferably, *in vitro* or more preferably, *in vivo*. Alternatively a benign, transformed organism may be introduced into the patient, the organism preferably expressing both Tat and the antigen against which it is desired to raise an immune response, but at least expressing Tat. The organism is suitably a virus or bacterium, and may be an attenuated form of an organism against which it is desired to stimulate an immune response.

Preferably, Tat is expressed *in situ* under the control of an inducible promoter, such that expression of Tat in the target cell can be induced by the user at or near the same time as administration or expression of the antigen. Suitable inducible promoters are well known but include those activated by physical means such as the heat shock promoter, although this is not generally preferred, or those activated by chemical such as IPTG or Tetracycline (Tet). The Tet promoter system is particularly preferred as it allows both on/off control of expression and control of the level of expression.

For Tat to be expressed in the target cell, it is preferable that the vaccine comprises an expression sequence. This sequence, element or vector is capable of expressing Tat in said cell and may be a viral vector, preferably attenuated, preferably an adenoviral vector capsid that can induce expression of Tat in the target cell. Other methods of inducing gene expression in target cells are known in the art and are also mentioned below.

It is, therefore, also preferred that the antigen is administered with a factor that controls or induces expression of Tat from an inducible promoter.

Alternatively, it is preferred that the antigen is expressed by administering a polynucleotide sequence encoding the antigen and that either a further polynucleotide sequence encoding Tat or a polynucleotide encoding a factor capable of inducing expression of Tat, is also provided, preferably substantially contemporaneously.

Expression *in situ* can be achieved by known methods of gene expression, such as the use of vectors, preferably viral vectors, that lead to expression of foreign DNA or RNA in a host. Preferably, polynucleotides encoding Tat are delivered and expressed by adenoviral or attenuated HIV systems. Alternatively, the polynucleotides can be delivered and expressed by methods such as the use of so-called "gene-guns." Thus, it is preferred that Tat is endogenously expressed by the patient or vaccinee.

It will also be understood that where reference to Tat is made in the present application, it is intended to include mutants and fragments thereof, as also discussed herein, unless otherwise apparent to the skilled person. For instance, Tat may be wild type Tat or a shortened fragment of the Tat polypeptide sequence, or may be a tat mutant.

It is also preferred that Tat is exogenously produced and provided as a peptide. Preferably, Tat is administered as a precursor that may be cleaved *in vivo* to provide active Tat.

The patient or vaccinee is preferably a mammal, preferably an ape or monkey and mostly preferably a human. In an alternative embodiment, however, it is also preferred that the method, use or vaccine is not applied or administered to humans.

In a further aspect, the present invention also provides a vaccine or method for modulating proteosome subunit composition, preferably down-regulating particular subunit or subunits, preferably the LMP2 subunit.

In a still further aspect, the present invention also provides vaccines for eliciting an immune response against an antigenic substance having a plurality of epitopes, or for eliciting an immune response against a plurality of strains of an infectious organism, as discussed above. In one embodiment, the vaccine preferably comprises Tat, a biologically active equivalent, or a precursor therefor, the epitopes including both immunodominant and sub-dominant epitopes, and at least a part of the antigenic substance encoding or comprising a sub-dominant epitope thereof.

In a further embodiment, the vaccine preferably comprises Tat, a biologically active equivalent, thereof or a precursor therefore, and antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope.

As discussed above, Tat is preferably that shown in SEQ ID NO. 284. Furthermore, it is preferred that Tat and the antigen are provided as proteins or peptides.

The present invention also provides for the use of these vaccines, preferably to stimulate cross-strain immunity preferably in the treatment of disease, preferably HIV or influenza.

Preferably, the vaccine comprises a suitable vehicle for delivery of Tat and the antigen. Such vehicles are well known in the art.

DETAILED DESCRIPTION OF THE INVENTION

While a major application of the present invention is in the fight against viruses well-known to generate escape mutants, it is equally important for use against cancer and immunomediated diseases, where the ability to target sub-dominant epitopes is a significant advantage.

In addition, it will be understood that Tat may be used with a vaccine of the present invention to enable the identification of subdominant epitopes of any antigens, especially from disease forms. Identification may effectively take the form of subtractive analysis. An example of this might be to take identical animals, immunise

one against a tumour using standard vaccine, and another with a similar vaccine containing Tat, and then identifying what CTL epitopes the second animal had extra, by comparison with the first. Epitopes identified in this manner could be identified and isolated and used in vaccination programs, or in screening.

Subdominant epitopes are commonly found, not only in infectious organisms, but also in tumour and immunomediated disease antigens. Without being bound by theory, the presence of Tat appears to expose a greater number of regions of such antigens, thereby generating a more potent immune response against subdominant epitopes.

What is especially surprising is that it has been found that the use of Tat actually results in a reduction of CTL responses to immunodominant epitopes, despite the fact that these epitopes are still present and efficient. Indeed, the responses to these epitopes are reduced, while the response to subdominant epitopes becomes highly significant. It is particularly advantageous that the reduction of CTL responses against immunodominant epitopes is useful to avoid or reduce the formation of escape mutants.

In general, the effect of Tat appears to be to "equilibrate" CTL responses to the epitopes of an antigen, favouring a broader immunodominant/subdominant epitope-specific set of CTL responses to any given antigen. In addition, as stated above, the decrease of immunogenicity of immunodominant CTL epitopes may avoid escape mutants.

Previous vaccine studies in animals with Tat have shown the ability of this protein to stimulate an immune response, with the Tat protein acting both as an antigen and as an adjuvant. However, none of the studies, with Tat employed as an adjuvant or co-antigen, have shown or suggested the unexpected, unpredictable and striking ability of TAT to stimulate an immune response against the subdominant epitopes as demonstrated by the present invention. Essentially, no one would expect that Tat or, indeed, any other protein, to so significantly alter epitope generation and presentation.

Instead, we have now found that Tat can be used with a broad range of antigenic materials and that strong immune responses can be stimulated and observed against

subdominant epitopes which rarely generate a response, or even which generate no response in most individuals.

Subdominant epitopes may either be observed on antigenic materials also comprising dominant epitopes, or may be comprised in molecules not associated with dominant epitopes. The location of the subdominant epitope is not important to the present invention, although it is generally preferred that it be available for recognition by CTLs during the life cycle of this organism, in order that the immune response generated be able to affect the course of the infection. Preferably, the infection will be controlled or eliminated by the immune response generated.

Escape mutations of dominant epitopes are very common, and have been observed in most disease organisms. For example, influenza and HIV are both associated with a number of strains where the dominant epitopes have mutated. Such mutation is widely believed to be a defence mechanism and, while the mutation has little or no effect on the virus, it is sufficient that an individual immune to one strain of the organism has no effective immunity against the organism carrying the new mutation.

By way of contrast, there has been no evolutionary pressure on subdominant epitopes to be able to mutate, so that there has also been no pressure on these epitopes to become dissociated from active functions in the organism. Accordingly, subdominant epitopes are substantially conserved and unable to mutate without crippling the organism.

Thus, by employing Tat in the vaccines of the present invention, it is possible to generate immunity against subdominant epitopes which are otherwise obscured by the dominant epitopes, and thereby to generate immunity against the CTL epitopes, both subdominant and immunodominant, within a given antigen, permitting an immune response to be generated against not only the strain of pathogen immunised against, but also a majority of, if not all, future and existing variants of the pathogen.

As used herein, the term "variant" includes all forms of the antigen that may be presented by the disease form in question, provided that the antigen in question is still presented by the disease form. It is not readily possible to define the term more

closely, but it will be appreciated that escape mutations often mutate the immunodominant epitopes substantially, so that one form of variant might include those where only the immunodominant epitopes vary, but the remainder of the antigen remains 99% and preferably 100% unchanged.

Antigens of the present invention may be derived from a number of sources, including plants, parasites and fungi. However, it is preferred that the antigen or antigens are derived from bacteria, preferably Mycobacteria, preferably *Mycobacterium tuberculosis, M. bovis,* or *M. africanum*. The antigen may also, preferably, be derived from staphylococcal or bacilli bacteria.

It will be understood that the term derived from includes antigenic peptide fragments from an organism or virus or even the organism or virus itself, provided that an epitope is provided.

It is particularly preferred that the antigen is derived from viral sources, preferably herpes viruses or from the family of *pox viridae*, preferably from respiratory-disease causing viruses, especially Adenoviruses Picornaviruses, Rhinoviruses, Echoviruses and Coxsackieviruses, preferably those that are responsible for influenza.

Indeed about 30 to 50% of all colds are caused by one of the > 100 serotypes of rhinoviruses. At any one time only a few viruses are prevalent. Often a single virus is responsible during outbreaks in relatively closed populations, such as in a school or barracks. However, new disease-causing strains rapidly evolve which immunised or tolerant individuals are not capable of reacting to rapidly. The present invention helps to overcome this by increasing the number of sub-dominant epitopes, which can often be more highly conserved.

Preferably, the antigen is derived from Acute Respiratory Syndrome viruses, such as those leading to SARS.

Even more preferred is that the Antigen is derived from, is a fragment of or comprises an Immunodeficiency virus, preferably SIV, but most preferably HIV. Various HIV antigens are known, including Gag, Pol, Rev and Env. Preferably, the antigen is

derived from Gag or Env. Indeed, we have shown in the accompanying Examples that Tat is particularly useful against both Gag and Env as the number of epitopes Gag or Env recognised by a host immune systems is greater in the presence of Tat.

Preferably, the antigen is derived from a cancer or tumour, preferably, a bowel, stomach, lung, colon, or pancreatic tumour, or a melanoma.

It is also preferred that tat is useful in the treatment of immunomediated diseases, preferably allergies, asthma, bronchitis, autoimmune diseases, arthritis, gout and allied conditions, infections, gastroenteritis, dysentery, constipation, neoplasia or conditions associated with immunosuppression.

It is also preferred that Tat can be used a as co-antigen, that is, that Tat can be administered or expressed together with an antigen, Tat having the beneficial effect of increasing the number of epitopes, particularly sub-dominant epitopes of the antigen.

A further advantage of administering or expressing Tat, preferably with a further antigen, is that an immune response will also be raised against Tat itself and it is envisaged that this could lead to an immune response to both the antigen and Tat.

Preferably, the antigen is administered as a protein or peptide. As discussed above, the protein or peptide may be modified such that it is protected from digestion or breakdown, for instance by use of glycosylation, provided that the protection can later be removed at the appropriate site, for instance the bloodstream, for instance by blood-borne glycosylases or glycosylases administered to the blood.

Preferred routes of administration are discussed below, include oral, intravenous, intramuscular, or subcutaneous. Preferably, the antigen is provided in a form adapted for such delivery, and may be in the form of a tablet, pill, suppository or liquid suitable for injection, or it may be contained with polysaccharide spheres or particles or nanoparticles.

The vaccines of the present invention comprise Tat, a biologically active equivalent mutant or fragment thereof, thereof or a precursor therefor. As shown in the

accompanying Examples, oxidised Tat has little or no effect, so that it is important to retain the biological activity of Tat. Within this requirement, it is possible to alter the Tat molecule, provided that the enhanced proteolytic activities of the immuno-proteasomes is conserved. This level of activity should be at least 30% of that shown in the accompanying Examples for each proteolytic activity. Preferably, the proteolytic activity should be at least 50% of the activity shown in the Examples, and preferably 80% and more preferably at least 90% of the activity shown in the Examples. For the avoidance of doubt, where more than one level of increased proteolytic activity is demonstrated in the accompanying Examples, then the above definition applies to the least of the listed activities, but may apply to any of the others, and preferably applies to the greatest activity.

Tat contains four domains. The acidic domain (amino acid residues 1 to 21) is important for interaction with cellular proteins. The cysteine rich region (amino acid residues 22 to 37) corresponds to the transactivation domain and is highly conserved among primary isolates. For example, replacing cysteine 22 with a glycine residue, leading to a so-called Tat22 mutant, abolishes the ability of Tat to transactivate the HIV-LTR. Likewise, the core domain (amino acid residues 38 to 48) is highly conserved, and simple substitution of lysine 41 with a threonine also incapacitates the transactivating ability of Tat on HIV-LTR. The fourth domain is the basic domain (amino acid residues 49 to 57), which is rich in arginine and lysine, and is responsible for the nuclear localisation of Tat, binding specifically to target RNA. This fourth domain is also responsible for binding extracellular Tat to heparin and heparansulphate proteoglycans. The carboxy terminal region is not necessary for LTR transactivation, but contains an arginine-glycine-aspartic acid sequence (RGD), common to extracellular matrix proteins, responsible for the interaction and binding of Tat to the integrin receptors $\alpha_5 \beta_1$ and $\alpha v \beta_3$.

Mutation of any of the domains or the carboxyl terminal is encompassed within the present invention, provided that the resultant biologically active Tat is still sufficient to stimulate the proteolytic activity of the immunoproteasomes as defined above.

In place of Tat, or a biologically active equivalent thereof, it is possible to use a nucleic acid sequence encoding either Tat or a biologically active equivalent thereof. In particular, the Tat, if not administered as part of the vaccine, may be expressed *in situ*, either by microbial systems in the vaccine, or as a result of administration of suitable expression sequences to the patient.

Likewise, the antigen comprised in the vaccine may also be presented in the form of a nucleic acid sequence encoding the antigen, or in the form of the original or a partially digested version of the original antigen, or a peptide. Although the subdominant epitope may be incorporated *per se* within the vaccine, this is not generally necessary when Tat is used, as Tat is capable of causing the presentation of subdominant epitopes by MHC-I.

It is convenient simply to incorporate antigenic material from the desired organism into the vaccine, as the unique activity of Tat is sufficient to decrease the immune response to the dominant epitope while substantially increasing the immune response to the subdominant epitope or epitopes. Although it is not essential to completely inactivate the infectious organism for the purposes of the vaccine, it is highly preferred, and this may be achieved by heat treatment or attenuation, for example. Further purification may be effected, if desired, such as by HPLC, ultrafiltration or centrifugation. Immunosorbent columns may also be used to separate ingredients.

The vaccines of the present invention may be used both for priming and boosting an immune response, and it is generally preferred that the composition of both the primary vaccine and booster is the same, although this is not necessary, provided that both the primary vaccine and the booster are to the same species of infectious organism, as the subdominant epitopes are conserved within the species.

Subsequent boosters may be applied as recommended by the skilled physician, and it is an advantage that it is not necessary to use the current virulent strain of an infectious organism to provide an effective vaccine.

The present invention further provides a method for providing an immune response against a plurality of strains of an infectious organism, comprising administering a vaccine comprising:
antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope; and
Tat, a biologically active equivalent thereof or a precursor therefor.

Preferably, Tat is as disclosed in SEQ ID NO. 284 or is a mutant and/or fragment thereof, as discussed elsewhere herein, and references to Tat and associated terms should be construed accordingly, in the absence of any indication to the contrary.

It is preferred that the infectious organism be a disease organism, and it is particularly preferred that the organism be a virus, although this is not necessary. Suitable sources of antigens are well known and are further discussed above.

Vaccines for use in the present invention may be provided in any suitable form and may be for administration by any suitable route. For example, vaccines of the invention may be provided intravenously, intramuscularly, intraperitoneally, subcutaneously, transdermally or in the form of eyedrops, or even as pessaries or suppositories.

Vaccines of the present invention may comprise any suitable ingredients in addition to the Tat and antigen ingredients, including, for example, stabilisers, buffers, saline, and isotonicity agents for injections, and any suitable ingredients, such as emulsifying agents and solid vehicles for applications such as pessaries and suppositories. Antibacterial and sterilising agents may also be employed, if desired.

In accompanying Example 1, we show that native HIV-1 Tat protein, an early product of HIV-infected cells, modifies the subunit composition and the activity of proteasomes. In particular, proteasomes in cells of B and T cell origin, either expressing endogenous Tat or exposed to a biologically active Tat protein, show up-regulation of LMP7 and MECL1 subunits and down-modulation of the LMP2 subunit. Strong down-regulation of the LMP2 subunit was shown to occur in splenocytes isolated from mice after treatment with native Tat but not with oxidised Tat protein,

and selective down-regulation of LMP2 by viral gene products has been reported[35,36]. It is known that the substitution of standard β-subunits with IFN-γ-inducible subunits alters the hydrolytic activity of proteasomes towards tri- and tetra-peptides, and the quality of the peptide products derived from polypeptides[10,23-25]. We demonstrate here that changes in proteasome subunit composition induced by Tat result in the increase of all three major proteasome proteolytic activities [10,23-25].

Perturbation of the proteasome system by viral infection, cell transformation or pharmacological treatments is often a key event in the modulation of the immune response to pathogens[37-44], since proteasomes play a pivotal role in the generation of the majority of antigenic peptides presented by MHC class I molecules[8]. In particular, immunoproteasomes are very efficient for the generation of specific CTL epitopes, and it has been shown that substitution of standard β-subunits with LMP2, LMP7 and MECL1 subunits improves the production of peptide antigens with the correct C termini for binding to MHC class I[45-48]. By way of contrast, there is evidence, both in humans and mice, that the presence of LMP2 may inhibit the presentation of specific peptide antigens[12,49,50].

We now show that the variations in proteolytic activity of proteasomes in Tat-expressing cells or in cells exposed to Tat protein correlate with a different presentation of EBV-derived epitopes, for example. In the accompanying Example, we show that Tat decreases the presentation of two immunodominant CTL epitopes (IVT and AVF) presented by HLA-A11 molecules, and increases the presentation of two subdominant epitopes (YLQ and CLG) presented by HLA-A2. HLA-A2-associated peptides present a valine at the C terminus, and it has been demonstrated that the β1 subunit, replaced by LMP2 in the immunoproteasomes, is responsible for cleavage beyond acidic residues and beyond residues with branched chains, such as valine[10,51].

It is, therefore, preferred that the present invention stimulates the down-regulation or replacement of LMP2 subunits, and preferably an up-regulation of β1 subunits in proteasomes. It is also preferred that the present invention stimulates an increase in

the number of peptides cleaved at Valine. Also preferred is a vaccine or method for increasing the number of epitopes recognises, particularly sub-dominant epitopes.

Preferably, Tat, its equivalent mutant or fragment, or precursor, is capable of down-regulating levels of LMP2 in the intended recipient of the vaccine.

Proteasomes in Tat-expressing/treated cells present low levels of LMP2 and higher post-acidic activity, compared with untreated cells or with cells that do not express Tat, which may account for the greater enhancement in the generation and presentation of YLQ and CLG CTL epitopes that present a C-terminal valine[10,51]. We also show that proteasomes from Tat-expressing cells are very efficient in the degradation of a CLG peptide precursor and can generate immunogenic peptide fragments therefrom, in contrast to proteasomes isolated from control cells.

A similar phenomenon was observed for an HLA-A2 presented epitope expressed in melanoma cells[12], suggesting that the presence of LMP2 may particularly affect the range of peptides presented by some HLA class I alleles, such as HLA-A2. This suggests that the presence of LMP2 is critical for the generation of CTL epitopes. Indeed, it has been demonstrated that influenza-specific CTL responses to the two most dominant determinants decrease in LMP2 knock-out mice, whereas responses to two subdominant epitopes are greatly enhanced[50]. Similarly, we demonstrated that the Tat-dependent LMP2 down-modulation induces changes in the hierarchy of CTL responses directed to Ova-derived CTL epitopes.

What we have demonstrated, for the first time, is that Tat increases CTL responses directed to subdominant epitopes and decreases those directed to the immunodominant SII peptide.

This is achieved by modifying the catalytic subunit composition and activity of immunoproteasomes in B and T cells which either express Tat, or have been treated with biologically active exogenous Tat protein. This results in modulation of the *in vitro* CTL epitope hierarchy. In particular, both intracellularly expressed and exogenous native Tat protein increase the major proteolytic activities of the proteasome by up-regulating LMP7 and MECL1 subunits and by down-modulating

the LMP2 subunit. This results in a more efficient generation and presentation of subdominant CTL epitopes

Decreasing the presentation of immunodominant epitopes, accompanied with an increase in the presentation of subdominant epitopes, is particularly beneficial for the elimination of virally infected cells, given that it is well established that immunodominant epitopes are very prone to mutation and to viral-escape, while subdominant epitopes are more stable while being capable of inducing protection[52].

Thus, Tat protein is useful to drive the induction of MHC-I restricted immune responses, broadening the spectrum of the epitopes recognised and increasing the chances to prevent the appearance of viral escape.

As mentioned above, we have shown that the presence of Tat results in a more efficient generation and presentation of subdominant CTL epitopes. Since the amount of MHC-I/epitope complexes is crucial in determining the presence and the strength of epitope-specific CTL responses and to verify the biological relevance of these findings for vaccination strategies, we went on to evaluate epitope-specific CTL responses against ovalbumin in mice vaccinated with both Tat and ovalbumin.

Surprisingly, we also found that Tat decreases CTL responses directed to the immunodominant epitope while inducing those directed to subdominant and cryptic T-cell epitopes that were not present in mice vaccinated with ovalbumin alone.

This finding suggests that Tat favours the generation of CTL responses directed to "weak" CTL epitopes and could therefore be used as a tool to increase CTL responses to heterologous antigens.

In addition, we found that a mutated form of the HIV-1 Tat protein, carrying glycine instead of cysteine 22 (Tatcys22) (SED ID NO 286), like wild-type Tat, modifies the subunit composition of proteasomes. The Tatcys22 mutant, in contrast to wild-type Tat, has no effect on the transactivation of the HIV-1 LTR, and does not induce reactivation of latent infection. This is particularly advantageous as administration or expression of biologically inactive tat may be appropriate in some circumstances.

Thus, we have also shown that the Cys residue at position 22, although key to the function of wild type Tat, is not required for Tat's effect on proteosome subunit composition.

It is a particular advantage of the TatCys22 mutant (SED ID NO 286) that it shows an improved effect compared to wild type Tat. That is, the TatCys22 mutant has actually been shown to increase the number of subdominant epitopes processed and, thereby, presented.

Indeed, our results in Experiment 3 show that T cell responses induced by vaccination with Gag+Tatcys22 are directed to 11 different T cell epitopes, 7 more than mice immunised with Gag alone, and 4 more than mice immunized with Gag and wild-type Tat.

We also demonstrate that peptide 47-86, derived from the wild-type Tat protein, is sufficient to down-modulate the LMP2 subunit.

Therefore, mutated forms of Tat, or Tat-derived peptides, represent an important alternative to the use of wild-type Tat in vaccination strategies aimed at increasing epitope-specific T cell responses directed to heterologous antigens.

We exploited the effect of Tat (both wild-type Tat protein and mutant Tatcys22 protein) on T cell responses against structural HIV gene products *in vivo*. We showed that, surprisingly, Tat increases the number of CTL epitopes within HIV Gag and Env antigens.

Balb/C mice were immunised with HIV-1 Gag or Env protein antigens, either alone or in combination with wild-type Tat or mutant Tatcys22. We found that both wild-type Tat and mutated Tatcys22 increase the number of epitope-specific T cell responses against Gag and Env antigens. In particular, we demonstrated that mice vaccinated with Gag, in combination with wild-type Tat or with the mutant Tatcys22, responded to 7 or 11 T cell Gag-derived epitopes respectively, in contrast to mice vaccinated with Gag alone, which responded to 4 T cell Gag-derived epitopes. Similarly, mice vaccinated with Env, in combination with wild-

type Tat or with the mutant Tatcys22 responded to 12 Env-derived pools of epitopes, in contrast to mice vaccinated with Env alone, which responded to 8 T cell Env-derived peptide pools.

Our results show that Tat is not only an antigen but also an adjuvant capable of increasing T cell responses against heterologous antigens. Therefore, the Tat protein, as well as mutant Tatcys22, represents an important tool in HIV-1 vaccine strategies aimed at broadening the spectrum of the epitopes recognized by T cells.

Thus, Tat is a useful tool for inducing epitope-specific CTL responses against HIV antigens and can be used as co-antigen for the development of new vaccination strategies against AIDS.

DESCRIPTION OF THE DRAWINGS

In the following Examples reference is made to the accompanying Figures, in which:

Figure 1 shows Tat DNA and RNA analysis in transduced MIN and MON LCL's
Fig 1A: PCR analysis was performed on genomic DNA (200 ng) from transduced and not transduced MIN- and MON-LCL's, using Tat1 and Tat2 primers. pCV-*tat* plasmid DNA (0.1 ng) was amplified as positive control. Amplified product is 240 bp. Molecular weight marker (MW): GeneRuler 100 bp DNA Ladder (MBI Fermentas).
Fig 1B: RT-PCR analysis was performed on cDNA from transduced and not transduced LCL's, using Tat1 and Tat2 primers. pCV-Tat plasmid DNA (0.1 ng) was amplified as positive control. Molecular weight marker (MW): GeneRuler 100 bp DNA Ladder (MBI Fermentas). Panel c: Northern blot analysis: total RNA (40 μg), purified from transduced and not transduced MIN- and MON-LCL's, was hybridised with ^{32}P-labeled Tat PCR product as probe. The positions of 28S and 18S rRNA's, as molecular size markers, are indicated.

Figure 2 shows Expression of proteasome subunits in cells transduced with HIV-1 *tat* gene

Left panel: Equal amount of total proteins from cell lysates from MIN and MON LCL's transduced with pBabeP (MIN-0 and MON-0) or with pBabeP-Tat (MIN-Tat and MON-Tat) were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with mAbs or polyclonal anti-sera specific for the α-2 subunit, PA28α, LMP2, LMP7 and MECL1. Right panel: The intensity of specific bands was measured by densitometry. Data are expressed as % increase in optical densities of Tat expressing cells relative to control cells. One representative experiment out of four performed is shown.

Figure 3 shows Activity of proteasomes purified from cells transduced with the HIV-1 *tat* gene

Purified proteasomes from cell lysates of MIN and MON LCL's transduced with pBabeP (MIN-0 and MON-0) or with pBabeP-Tat (MIN-Tat and MON-Tat) were tested for chymotryptic-like, tryptic-like and post-acidic activities using Suc-LLVY-AMC, Boc-LRR-AMC and Ac-YVAD-AMC as substrates, respectively. Peptide substrates (100 μM) were incubated with 5 μg of purified proteasomes at 37°C for 30 min. Data are expressed as arbitrary fluorescence units. One representative experiment out of three performed is shown.

Figure 4 shows Expression of proteasome subunits in cells treated with the HIV-1 Tat protein

Left panel: Equal amount of total proteins from cell lysates from MIN and MON LCL's treated with the indicated concentrations of the native Tat protein were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with mAbs or polyclonal anti-sera specific for the α-2 subunit, LMP2, LMP7 and MECL1. Right panel: The intensity of specific bands was measured by densitometry. Data are expressed as % increase in optical densities of Tat expressing cells relative to control cells. One representative experiment out of three performed is shown.

Figure 5 shows Expression of the LMP2 subunit in splenocytes isolated from mice treated with Tat protein

Mice were treated with native Tat protein (panel a) or with oxidized Tat (panel b) and after 3 i.m. treatments, splenocytes were isolated and lysed. Equal amount of total

proteins from cell lysates were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with an antibody specific for LMP2. The intensity of LMP2 bands was evaluated by densitometry and normalised to the correspondent expression of proteasomes evaluated with a polyclonal sera specific for α-subunits. Data are expressed as % increase in optical densities compared to the mean of LMP2 expression in control splenocytes from 6 untreated mice.

Figure 6 shows CTL killing of cells transduced with the HIV-1 *tat* gene

The HLA-A2, -A11 positive MIN and MON LCL's transduced with pBabeP (MIN-0 and MON-0) or with pBabeP-Tat (MIN-Tat and MON-Tat) were used as target in cytotoxic assays of CTLs specific for the HLA-A11 presented, EBNA4-derived IVT and AVF epitopes, the HLA-A2-presented Lmp1-derived YLQ epitope, and the HLA-A2-presented Lmp2-derived CLG epitope, respectively. Results are expressed as % specific lysis. One representative experiment out of three performed is shown.

Figure 7 shows CTL killing of cells treated with exogenous HIV-1 Tat protein

The HLA-A2, -A11 positive MIN LCL's, treated or not with Tat, were used as target in cytotoxic assays of CTLs specific for the HLA-A11 presented, EBNA4-derived IVT and AVF epitopes, the HLA-A2-presented Lmp1-derived YLQ epitope, and the HLA-A2-presented Lmp2-derived CLG epitope. Results are expressed as % specific lysis. One representative experiment out of three performed is shown.

Figure 8. In vitro degradation of a CLG epitope precursor by proteasomes purified from Tat-expressing cells.

Panel A: the CLG+5 peptide was incubated with proteasomes purified from MIN-Tat or from MIN-0 LCLs. The precursor degradation was followed at different time points and the degradation of CLG+5 was evaluated by HPLC analysis. Data are expressed as % degradation. The mean of the results from three independent experiments is shown.

Panel B: the digestion products obtained after 120 min of degradation were purified by HLPC, the indicated fractions were collected and tested by IFN-γ Elispot for their capacity to activate CLG-specific CTLs. Data are expressed as spot-forming cells

(SFC) per 10^6 cells. The mean of the results from three independent experiments, performed in triplicates, is shown.

Figure 9 shows Ova-specific CTL responses in mice vaccinated with Ova and Tat protein

Mice were vaccinated with Ova alone or with Ova and Tat protein. After 2 immunisations, fresh splenocytes were pooled and tested in cytotoxicity against EL4 cells pulsed with SII, KVV, or CFD peptides. Data are expressed as % specific lysis calculated by subtracting lysis of untreated EL4 cells (always below 10%). Mean of two independent experiments performed in triplicate.

Figure 10 shows Expression of proteasomes in Jurkat cells expressing the HIV-1 *tat* gene.

Fig 10A: equal amounts of purified proteasomes (1 μg) from Jurkat cells transfected with the vector alone (JSL3-0), or with the *tat* gene (JSL3-Tat), were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with mAbs or polyclonal anti-sera specific for α-2 subunit, LMP2, LMP7 and MECL1. One representative experiment out of the four performed is shown.

Fig 10B: The intensity of specific bands was measured by densitometry. Data are expressed as % increase in optical densities of specific bands detected in proteasomes purified from Tat expressing cells, relative to proteasomes from control cells. Mean +/- SEM of three independent experiments is shown.

Figure 11. Expression of proteasome subunits in Jurkat cells treated with the HIV-1 Tat protein.

Jurkat cells were treated for 12 (Fig 11 A) or for 24 (Fig 11B) hours at 37° C with 0.01, 0.1 or 1 μg/ml of the native Tat protein. Equal amounts of proteasomes (1 μg) were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with mAbs specific for the α-2 and LMP2 subunits. One representative experiment out of three performed is shown. The intensity of specific bands was measured by densitometry. Data are expressed in optical densities of specific bands detected in control cells (NT) and in Tat treated cells.

Figure 12 Enzymatic activity of proteasomes in Jurkat cells treated or untreated with 1 μg/ml of Tat for 24 hours.

Proteasomes (2.5 μg) purified from cell lysates of the indicated cell lines were incubated for 30 min at 37°C with Suc-LLVY-AMC, Boc-LRR-AMC and Ac-YVAD-AMC to evaluate chymotryptic-like, tryptic-like and post-acidic activities, respectively. Data are expressed as arbitrary fluorescence units.

Figure 13. Expression of proteasomes in Jurkat cells expressing wild-type or mutated HIV-1 *tat* genes.

Fig 13A: equal amounts of purified proteasomes (1 μg) from Jurkat cells transfected with the vector alone (Vect) or expressing wild-type Tat (Tat), mutant Tat22 (cys22 substituted with gly), mutant 37 (cys37 substituted with ser), or double mutant Tat22/37 were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with α-2 subunit- and LMP2 subunit-specific mAbs. One representative experiment out of the four performed is shown.

Fig 13B:the intensity of specific bands was measured by densitometry. Data are expressed as % increase in optical densities of specific bands detected in proteasomes purified from Tat expressing cells, relative to proteasomes from control cells.

Figure 14. The Tat-derived 47-86 peptide is sufficient to down-modulate the LMP2 subunit.

Jurkat cells were treated for 24 with 0,1 μg/ml with Tat or with peptides 1-38, 21-58 and 47-86 covering the wild-type sequence of Tat. Equal amounts of proteins from total cell lysates were fractionated by SDS-PAGE, transferred onto nitrocellulose filters, and probed with α-2 subunit- and LMP2 subunit-specific mAbs. One representative experiment out of the three performed is shown.

Figure 15. Ova-specific CTL responses in mice vaccinated with Ova alone or combined with the Tat protein.

Mice were immunized with 25 μg of ovalbumin alone or in combination with 5 and 10 μg Tat protein. After 2 immunizations, fresh splenocytes were pooled and tested in cytotoxicity against EL4 cells pulsed with SII, KVV, or CFD peptides. Data are

expressed as % specific lysis calculated by subtracting lysis of untreated EL4 cells (always below 10%). The mean of the results from three independent experiments, performed in triplicates, is shown.

Figure 16. Tat broadens the immune response against Env.

Mice (n=5) were immunized subcutaneously with Tat, TatCys22 and Env proteins alone or in combination, as described in materials and methods. Splenocytes (pools of spleens) of immunized mice were stimulated with pools of Env peptides, and tested for IFNγ production in the presence of each pool, medium alone (negative control) or Concanavaline A (positive control). Results are expressed as the number of spot forming units (SFU)/10^6 cells subtracted from the SFU/10^6 cells of the negative controls, as described in Example 3. Responses ≥ 50 SFU/10^6 cells are considered positive. Filled boxes mark reactive pools.

Figure 17. Gag-specific IFNγ T cell responses in mice vaccinated with Gag alone or combined with the Tat protein.

Mice (n=5) were immunized with 5 μg of Gag alone or in combination with 5 μg Tat protein. After 3 immunizations, fresh splenocytes were pooled, stimulated with the indicated pools of Gag peptides and tested for IFNγ release by Elispot assay. Results are expressed as SFU/10^6 cells subtracted from the SFU/10^6 cells of the negative controls, as described in Example 3. Responses ≥ 50 SFU/10^6 cells are considered positive.

Figure 18. Gag-specific IFNγ T cell responses in mice vaccinated with Gag alone or combined with the Tat protein.

Mice (n=5) were immunized with 5 μg of Gag alone or in combination with 5 μg Tat protein. After 3 immunizations, fresh splenocytes were pooled, stimulated with the indicated peptides of Gag peptides and tested for IFNγ release by Elispot assay. Results are expressed as SFU/10^6 cells subtracted from the SFU/10^6 cells of the negative controls, as described in Example 3. Responses ≥ 50 SFU/10^6 cells are considered positive.

Figure 19. Gag-specific IFNγ T cell responses in mice vaccinated with Gag alone or combined with the Tatcys22 protein.

Mice (n=5) were immunized with 5 μg of Gag alone or in combination with 5 μg Tatcys22 protein. After 3 immunizations, fresh splenocytes were pooled, stimulated with the indicated pools of Gag peptides and tested for IFNγ release by Elispot assay. Results are expressed as SFU/10^6 cells subtracted from the SFU/10^6 cells of the negative controls, as described in Example 3. Responses ≥ 50 SFU/10^6 cells are considered positive.

Figure 20. Gag-specific IFNγ T cell responses in mice vaccinated with Gag alone or combined with the Tatcys22 protein.

Mice (n=5) were immunized with 5 μg of Gag alone or in combination with 5 μg Tatcys22 protein. After 3 immunizations, fresh splenocytes were pooled, stimulated with the indicated peptides of Gag peptides and tested for IFNγ release by Elispot assay. Results are expressed as SFU/10^6 cells subtracted from the SFU/10^6 cells of the negative controls, as described in Example 3. Responses ≥ 50 SFU/10^6 cells are considered positive.

Figure 21. Peptide matrix setup for HIV-1 Env peptides

Pools 1-7 and pools 12-30 were designed so that 2 independent pools contain one peptide in common.

Pool 1, contains Env 1-Env 19 + Env 8771, 8772, 8773

Pool 2, contains Env 20-Env 38 + Env 8789, 8790, 8791

Pool 3, contains Env 39-Env 57 + Env 8805, 8806

Pool 4 contains Env 58-Env76 + Env 8822

Figure 22. Peptide matrix setup for HIV-1 Gag peptides

Shows matrix used for use with the Gag peptides in Example 3.

The invention is not to be limited by what has been particularly shown and described, except as indicated by the appended claims. Indeed, while the invention will now be illustrated in connection in connection with the following Examples, it will be understood that it is not intended to limit the invention to these particular

embodiments. On the contrary, it is intended to cover all alternatives modifications and equivalents, as may be included within the scope of the invention as defined by the appended claims.

EXAMPLE 1

The following methods were used in this and the following Examples.

Cells

PG13 murine amphotropic packaging cell line[54] was cultured in DMEM supplemented with 10% FCS. Jurkat T cell transfectants (pRPneo-c and pRPneo-c-Tat) [22] were cultured in RPMI 1640 medium, supplemented with 10% FCS and 800 μg/ml neomycin (Sigma). Lymphoblastoid cell lines (LCL) were established by *in vitro* infection of normal B-lymphocytes from healthy donors with the B95.8 strain of EBV. LCL's were cultured in RPMI 1640 medium supplemented with 10% FCS.

Plasmids

HIV-1 Tat cDNA sequence was amplified by PCR from pGEM-3-Tat plasmid[22] using primers Tat A: 5'-GGGGAATTCATGGAGCCAGTAGAT-3' (forward) (SEQ ID NO 271) and Tat B: 5'-CAAGAATTCCTATTCCTTCGGGCC-3' (reverse) (SEQ ID NO 272) (annealing temperature 57°C). The purified PCR product was sequenced and cloned into the EcoRI site of pBabePuro vector to generate pBabePuro-Tat[55].

Packaging cell lines

The pBabePuro and pBabePuro-Tat vectors were transfected into PG13 packaging cell line by the calcium phosphate method[56]. Transfected cells were cultured in selective medium containing 3 μg/ml of puromycin (Sigma). Production of recombinant retroviruses from selected cultures was tested by semiquantitative RT-PCR on cell-free DNase treated supernatants, using of primers PuroA/PuroB (PuroA: 5'-CGAGCTGCAAGAACTCTTCC-3' (forward) (SEQ ID NO 273), PuroB: 5'-AGGCCTTCCATCTGTTGCTG-3' (reverse) (SEQ ID NO 274); annealing temperature 57°C) and TatA/TatB respectively.

Cell transduction

MIN and MON LCL's were transduced with pBabePuro-Tat (MIN-Tat and MON-Tat) or pBabePuro (MIN-0 and MON-0) recombinant retroviruses by co-

cultivation with packaging cell lines using transwell-clear tissue culture membranes. Subconfluent PG13 pBabePuro and PG13 pBabePuro-Tat cells, grown in the lower chamber, were co-cultivated in the presence of 8 μg/ml polybrene (Sigma) with MIN or MON LCL's ($3x10^6$/well) added to the upper chamber in 2.5 ml of RPMI 1640 medium supplemented with 10% FCS. After 48 hrs of co-cultivation, cells were harvested from the membranes and grown in culture medium containing puromycin (0.3 μg/ml) for 6 weeks. All cell lines were characterised by DNA-PCR, RT-PCR and Northern blot analysis.

Characterisation of transduced cell lines

Total DNA was extracted from $5x10^6$ cells with the NucleoSpin Blood kit (Macherey-Nagel), as specified by the manufacturer. For amplification of Puromycin and Tat genes, primers PuroA/PuroB were used, under the conditions described above, and
Tat 1: 5'-gAAgCATCCAggAAgTCAgCC-3' (SEQ ID NO 275)
Tat 2: 5'-ACCTTCTTCTTCTATTCCggg-3' (SEQ ID NO 276) (annealing temperature 55°C).

RNA was extracted from cell-free supernatants of packaging cells, MIN and MON LCL's, MIN-0 and MON-0, MIN-Tat and MON-Tat cells ($5x10^6$) with NucleoSpin RNA II (Macherey-Nagel), as specified by the manufacturer. Total RNA (1 μg) was incubated with 20 mM $MgCl_2$ and 500 IU/ml pancreatic DNase I (Boehringer Mannheim) at 37°C for 1 hr and purified by phenol-chloroform. DNase digestion was repeated three times upon the addition of fresh DNase I.

RNA was reverse transcribed by using the random hexamer method with RT-PCR Systems (Promega) according to manufacturer's instructions. cDNA was tested by PCR using actin-specific primers :
forward: 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3'
(SEQ ID NO 277);
reverse: 5'-AGTCATAGTCCGCCTAGAAGCATTTGCGGT-3' (SEQ ID NO 278); annealing temperature 63°C). PCR's for Puromycin and Tat genes were performed as described.

Northern blotting

Equal amounts of total RNA's (40 μg) were electrophoresed onto formaldehyde-agarose gel (1.5%) for 12 hours, transferred onto nylon membranes (Hybond N; Amersham) and hybridised with DNA probe. Probes were randomly labelled with [^{32}P] dCTP, using the Prime-It II kit (Stratagene).

Western blotting

Equal amounts of proteins were loaded on a 12% SDS-PAGE gel and electroblotted onto Protran nitrocellulose membranes (Schleicher & Schuell, Keene, Hampshire, USA). The blots were probed with antibodies specific for α2, LMP2, LMP7, MECL1, and PA28α subunits (Affinity, Exeter, UK) and developed by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech, Uppsala, SW).

HIV-1 Tat protein

HIV-1 Tat from the human T lymphotropic virus type IIIB isolate (subtype B) was expressed in *E. Coli* and purified by heparin-affinity chromatography and HPLC as a Good Laboratory Practice (GLP) manufactured product as described previously[3]. The Tat protein was stored lyophilised at -80°C to prevent oxidation and reconstituted in degassed buffer before use, as described[2]. Different GLP lots of Tat were used with reproducible results and in all cases endotoxin concentration was below 0.05 EU/μg.

Purification of proteasomes

Cells ($5x10^7$) were washed in cold PBS and resuspended in buffer containing 50 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT, Sigma), 2 mM ATP, 250 mM sucrose. Glass beads equivalent to the volume of the cellular suspension were added and cells were vortexed for 1 min at 4°C. Beads and cell debris were removed by 5 minutes centrifugation at 1000 x g, followed by 20 minutes centrifugation at 10,000 x g. Supernatants were ultracentrifuged for 1 hour at 100,000 x g[44]. Supernatants were loaded into an affinity column containing an agarose matrix derivatised with the MCP21 mAb specific for the α2 subunit of the proteasome (Affinity, Exeter, UK). The column was washed, eluted with 25 mM Tris-HCl pH 7,5 containing 2 M NaCl, and 0.5 ml fractions were collected. Fractions containing

proteasomes were combined and dialysed against 25 mM Tris-HCl pH 7.5. Protein concentration was determined using BCA protocol (Pierce Chemical).

Enzyme assays

The fluorogenic substrates Suc-LLVY-AMC, Boc-LRR-AMC and Ac-YVAD-AMC were used to measure chymotrypsin-like, trypsin-like and post-acidic proteasome activities, respectively. Peptide substrates (100 μM) were incubated at 37 °C for 30 min with purified proteasomes in 75 μl of buffer containing 50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 500 μM EDTA pH 8.0, 1 mM dithiothreitol, and 2 mM ATP. Fluorescence was determined by a fluorimeter (Spectrafluor plus, Tecan, Salzburg, Austria) using an excitation of 360 nm and emission of 465 nm. Proteasome activity is expressed in arbitrary fluorescence units[11].

Synthetic peptides

All peptides were synthesised by solid phase methods[57]. Crude deprotected peptides were purified by HPLC to >98% purity. Structure verification was performed by elemental and amino acid analysis and mass spectrometry. Peptide stocks were dissolved in DMSO at a concentration of 10^{-2} M, kept at -20°C, and diluted in PBS before use.

Digestion of synthetic substrates

The synthetic peptide CLGGLLTMVAGAVW (CLG+5) (SEQ ID NO 279) was dissolved in DMSO at a concentration of 20 μg/μl. 500 μg of synthetic peptide were incubated with 127 μg of purified proteasomes in 300 μl of buffer (25 mM Tris HCL pH 7.4, 5 mM $MgCl_2$, 500 μM EDTA pH 8.0, 1 mM DTT, 2 mM ATP) at 37°C. At the indicated time points, 60 μl of sample were collected and the reaction was stopped by adding 2 volumes of ethanol at 0°C.

Digestion mixtures were centrifuged at 5000 rpm for 5 minutes. 80 μl of supernatant were collected and peptide digests were separated by reverse-phase HPLC at the flow rate of 0.7 ml/min, as follows: linear gradient of 0-100% of solution B (acetonitrile 100% with 0.1% TFA) for 25 min, followed by linear gradient of 0-100% for solution A (water 100% with 0.1% TFA) for 5 min. The fractionation was simultaneously

monitored at 210 and 280 nm. Fractions were collected every 30 seconds and stored at +4°Cand tested in ELISPOT.

Generation of CTL cultures

HLA A11-restricted EBV-specific CTL cultures reacting against the EBNA4-derived IVTDFSVIK (SEQ ID NO 280) (IVT) and AVFSRKSDAK (SEQ ID NO 281) (AVF) epitopes, corresponding to amino acid 416-424 and 399-408[27], were obtained by stimulation of monocyte-depleted PBL's from the HLA-A11-positive EBV-seropositive donor MC with the autologous B95.8 virus-transformed LCL. HLA A2-restricted EBV-specific CTL cultures reacting against the Lmp2-derived CLGGLLTMV (SEQ ID NO 282) (CLG) epitope, corresponding to amino acid 426-434[28], and the Lmp1-derived YLQQNWWTL (SEQ ID NO 283) (YLQ) epitope corresponding to amino acid 159-167[29], were obtained by stimulation of monocyte-depleted PBL's from the HLA-A2-positive EBV-seropositive donor RG with peptide-pulsed T2 cells, as previously described[32]. The first stimulation was performed in RPMI 1640 medium containing 10% FCS. A second and a third stimulation were performed in the same conditions on day 7 and day 14. Starting from day 8 the medium was supplemented with 10 U/ml rIL-2 (Chiron, Milan, Italy).

Cytotoxicity assay

Target cells were labelled with $Na_2{}^{51}CrO_4$ for 90 min at 37° C. Cytotoxicity tests were routinely run at different effector : target ratios in triplicate. Percent specific lysis was calculated as
100x(cpm sample - cpm medium)/(cpm Triton X-100 - cpm medium)[27]. Spontaneous release was always less than 20%.

ELISPOT assay

CTLs ($4x10^4$ cells) were plated in triplicate on microplate 96-wells unifilter (Whatman) previously coated with 100 µl of an anti IFN-γ mAb (Endogen, Woburn, MA) overnight at 4°C. CTLs were incubated with medium alone as a negative control, with phytohaemagglutinin (PHA) as a positive control, or with 20 µl of each HPLC fractions derived from the *in vitro* digestion by proteasomes of epitope precursors.

Plates were incubated for 24 h at 37°C, 5% CO_2 and then washed three times with PBS and three times with washing buffer (PBS 0,05% Tween 20) before 100 µl of biotinylated anti-IFN-γ MAb (1 µg/ml; Endogen, Woburn, MA) were added, and incubated at 37°C for 60 min. After the plates were washed again, HRP-conjugated streptavidin (Endogen, Woburn, MA) was added and the plates were incubated at room temperature for 45 min. Wells were washed , and individual IFN-γ producing cells were detected using AEC chromogen kit (Sigma, Saint Louise, Missouri). IFN-γ-secreting T cells were counted by direct visualisation. The number of specific IFN-γ-secreting T cells, expressed as spot-forming cells (SFC) per 10^6 cells, was calculated by subtracting the negative control value. Negative control values were always <500 SFC per 10^6 input cells.

Animal use was according to national and institutional guidelines. Seven-to-eight week old female Balb/c mice (Nossan, Milan, Italy) were injected with native monomeric biologically active Tat protein (1 µg) resuspended in degassed sterile PBS. Control mice were injected with oxidised Tat (1 µg) or with PBS alone. Samples (100 µl) were given by intramuscular (i.m.) injections in the quadriceps muscles of the posterior legs. Each experimental group consisted of three mice, and the experiment was repeated twice. Mice were boosted at days 11 and 20 after the first injection. Seven days after the last injection, animals were anaesthetised intraperitoneally (i.p.) with 100 µl of isotonic solution containing 1 mg of Inoketan (Virbac, Milan, Italy), and 200 µg Rompun (Bayer, Milan, Italy) and sacrificed to collect spleens. Mononuclear cells from individual spleens were purified using cells strainers, resuspended in PBS containing 20 mM EDTA, and treated with a red blood cells lysis buffer for 4 minutes at room temperature. Cells were washed twice in PBS, lysed and used for western blot analysis as described above.

Seven- to eight- week old female C57BL/6 mice ($H\text{-}2^b$) (Nossan) were injected with 25 µg ovalbumin (Sigma, St. Louis, MO) alone or in combination with native monomeric biologically active Tat protein (5 and 10 µg) and resuspended in degassed sterile PBS in Freund's adjuvant (CFA for the first injection, and IFA for subsequent injections). Control mice were injected with PBS alone in Freund's adjuvant.

Samples (100 µl) were given by subcutaneous (s.c.) injection in one site in the back. Each experimental group consisted of five mice, and the experiment was repeated twice. Mice were boosted at day 24. Two weeks after the last injection, animals were anaesthetised i.p., as described above, and sacrificed to collect spleens. Mononuclear cells from individual spleens were purified as described above, pooled and tested in cytotoxic assay against peptide-pulsed EL4 cells.

Endogenously expressed Tat modulates proteasome composition and activity
To evaluate proteasome expression in the presence of endogenous Tat, lymphoblastoid cell lines (LCL) expressing Tat (MIN-Tat and MON-Tat) were prepared by retroviral transduction and assayed (Figure 1) for the presence of integrated plasmids and for the expression of Tat RNA as compared to vector transduced cells (MIN-0 and MON-0).

The level of expression of proteasomes was then analysed by Western blot analysis in both Tat-expressing cells as compared to control cells. No difference in proteasome expression was detected in these cells by the use of a monoclonal antibody specific for the α2-subunit (Figure 2). Since LCL's constitutively express immunoproteasomes[11], we then evaluated the expression of the IFNγ-inducible PA28α regulator and of the catalytic β subunits LMP2, LMP7 and MECL1. Both Tat-Tat showed no differences in the expression of PA28α regulator as compared to control cells. In contrast, a marked down-regulation of LMP2 and up-regulation of LMP7 and MECL1 subunits were observed in both Tat-expressing cell lines as compared to the control cells (Figure 2). A similar increase of LMP7 and MECL1, and decrease of LMP2 were detected in a Jurkat T cell line stably transfected with Tat[22] as compared with Jurkat cells transfected with the empty control vector (Fig 10, Example 2). These findings demonstrate that the subunit composition of immunoproteasomes is affected by the endogenously expressed HIV-1 Tat protein.

To investigate whether the differences in subunit composition detected by Western blot analysis correlated with differences in enzymatic activity, we analysed the cleavage specificity of equal amount of proteasomes isolated from MIN-Tat and MON-Tat cells or control cells. We tested chymotryptic-like, tryptic-like, and post-

acidic activities using Suc-LLVY-AMC, Boc-LRR-AMC and Ac-YVAD-AMC as substrates, respectively. All three enzymatic activities were higher using proteasomes purified from cells expressing Tat as compared to activities of proteasomes purified from control cells (Figure 3). This observation is in agreement with the results of expression of the three catalytic subunits, since it has been demonstrated that expression of LMP7 and MECL1 is associated with increased chymotryptic and tryptic activities, whereas LMP2 expression is associated with a decreased post-acidic activity[10,23-25]. Indeed, LCL's expressing Tat showed an increased expression of LMP7 and MECL1 and a decreased expression of LMP2 when compared to control cells.

Exogenous biologically active Tat modulates proteasome composition and activity

To test the effect on proteasomes in cells after the up-take of exogenous Tat protein, MIN and MON LCL's were cultured in the absence or presence of increasing concentrations of biologically active Tat protein for 24 hours at 37°C. After treatment, the expression of the different subunits was analysed and compared to that of untreated cells. No difference in the expression of the α2-subunit was detected by Western blot analysis, suggesting that exogenous Tat does not alter the expression of proteasomes (Figure 4) as already observed in cell expressing endogenous Tat (Figure 2). However, treatment with 0.1-1 μg/ml of Tat determined a down-regulation of LMP2 and an up-regulation of LMP7 and MECL1 as compared to untreated cells (Figure 4). In addition, proteasomes isolated from MIN and MON LCL's treated with 0.1 μg/ml of Tat presented an increase of all three proteolytic activities as detected using specific fluorogenic peptides (data not shown). These results demonstrate that exogenous Tat protein alters the subunit composition and the proteolytic activity of immunoproteasomes in the cells, as demonstrated for cells endogenously expressing Tat.

***In vivo* modulation of proteasome composition by a biologically active Tat**

To evaluate the *in vivo* effect of Tat, we treated Balb/c mice with biologically active Tat or oxidised Tat. After 3 consecutive treatments, splenocytes were isolated and total cell lysates tested for the expression of IFN-γ inducible catalytic subunits.

Splenocytes isolated from all mice treated with native Tat demonstrated the down-regulation of LMP2, while no effect was observed in splenocytes isolated from mice treated with oxidised Tat (Figure 5). These results demonstrate that Tat regulates *in vivo* the subunit composition of proteasomes.

Tat modifies the generation of CTL peptide epitopes derived from EBV latent antigens

Since proteasomes play a key role in the generation of CTL epitopes, we investigated the effect of proteasome variation induced by Tat on the generation and presentation of CTL epitopes in LCL's expressing endogenous Tat or exposed to Tat protein.

LCL's express the total set of EBV latent antigens, including nuclear antigens (EBNA) 1, 2, 3, 4, 5, 6 and latent membrane protein (LMP) 1 and 2. These antigens, except for nuclear antigen 1, are targets of cytotoxic T lymphocytes and a large number of CTL epitopes have been identified[26]. In this set of experiments we evaluated: the immunodominant IVT and AVF HLA-A11-presented epitopes, that derive from the EBNA4 antigen[27], and of the subdominant YLQ and CLG epitopes, two HLA-A2-presented epitopes derived from the latent membrane protein 1 (Lmp1) and 2 (Lmp2), respectively[28,29]. It has been recently shown that the generation of these epitopes depends on proteasome activity[30,31]. To this purpose, the HLA-2 and -A11 positive MIN LCL and the HLA-A2 positive MON LCL, transduced or not with Tat, were tested as target in cytotoxic assays using CTL cultures specific for the IVT, AVF, CLG and YLQ epitopes (Figure 6). As demonstrated previously [27], IVT- and AVF-specific CTLs efficiently lysed A11-matched LCL, whereas lower levels of specific killing were obtained with YLQ- and CLG-specific CTLs[28,29,32]. This is due to the poor expression of these two HLA-A2-presented epitopes at the cell surface of EBV-infected B cells[26].

The expression of endogenous Tat caused a decrease of IVT- and AVF-specific CTL killing and an increase of YLQ- and CLG-specific killing as compared to control cells transduced with the empty vector (Figure 6). The HLA-A11-negative MON LCL, either expressing Tat or the empty vector, were not recognised by IVT- and AVF-specific CTL cultures.

In a second set of experiments we evaluated CTL sensitivity of MIN LCL untreated or treated with 0.1 μg/ml of the Tat protein for 24 hours. In agreement with the results of the previous experiments, LCL's treated with Tat were less sensitive to IVT- and AVF-specific CTL killing but were lysed at higher efficiency by YLQ- and CLG-specific CTLs (Figure 7).

These findings suggest that the effect of Tat on proteasome composition and activity results in changes of epitope presentation at the surface of virally infected cells.

Efficient *in vitro* generation of the CLG epitope by proteasomes purified from Tat-expressing cells

To test whether proteasomes from Tat-expressing cells generate the CLG epitope with more efficiency, we analysed the *in vitro* degradation of a CLG peptide precursor that contains 5 amino acids at the C-terminus (CLG+5) corresponding to the wild-type sequence of the LMP2 antigen. The *in vitro* assay was performed using proteasomes purified from MIN-Tat and proteasomes purified from MIN-0. The precursor degradation was followed at different time points and evaluated by HPLC analysis. We found that proteasomes isolated from Tat-expressing cells degraded the CLG+5 peptide precursor more efficiently than proteasomes purified from cells expressing the empty vector (Figure 8a).

To characterise the digestion products, we separated the digests obtained at different time points by HPLC. All fractions were collected and used in ELISPOT to activate CLG-specific CTLs. Fractions purified from digests obtained after 30, 60, and 90 min of incubation did not activate CTL responses (not shown). Only HPLC fractions 4 and 8 obtained after 120 min of degradation with proteasomes purified from MIN-Tat did stimulate CLG-specific CTL responses. This demonstrates that these fractions contain the CLG epitope or a longer but immunogenic epitope. A weak CLG-specific CTL response was also observed in HPLC fraction 8 obtained after 120 min of degradation using proteasomes isolated from MIN-0. These results demonstrate again that proteasomes purified from Tat-expressing cells exhibit a different proteolytic activity and that generate with more efficiency the immunogenic CLG peptide.

***In vivo* modulation of CTL responses by Tat**

As shown above, Tat alters, both *in vitro* and *in vivo*, the subunit composition of proteasomes which, in turn, modulates the presentation of EBV-derived CTL epitopes at the cell surface of EBV-infected B cells. Down-modulation of two immunodominant CTL epitopes and up-regulation of two subdominant CTL epitopes (Figures 5 and 6) was observed, suggesting that Tat, by altering the antigen processing machinery, may influence the epitope presentation on the antigen presenting cells affecting immunodominance and subdominance of CTL responses. Accordingly, we decided to evaluate the *in vivo* effect of Tat on the induction of epitope-specific CTL responses. We used as a model CTL responses directed to ovalbumin (Ova) on the K^b background. K^b-restricted CTL responses are directed to the immunodominant SIINFEKL (SII) (SEQ ID NO 268) epitope and to the subdominant KVVRFDKL (KVV) (SEQ ID NO 269) and cryptic CFDVFKEL (CFD) (SEQ ID NO 270) epitopes[33,34]. It has been shown that CTLs specific for KVV are not found upon immunisation of C57BL/6 mice with Ova and that the subdominance of the KVV and CFD epitopes was due to the presence of amino acidic sequences that flank the epitope and that affect the proteasome-mediated processing and the generation of KVV and CFD CTL epitopes[20,21]. To address whether Tat affects the *in vivo* generation of the K^b-restricted Ova-derived epitopes we vaccinated mice with Ova alone or in combination with Tat.

The presence of specific CTL responses directed to the three Ova-derived epitopes was evaluated on fresh splenocytes using EL4 target cells pulsed or not with the relevant CTL epitopes (Figure 9). Splenocytes isolated from mice immunised with Ova alone recognised target cells pulsed with the SII epitope but did not recognise cells pulsed with the KVV or CFD epitopes, thereby confirming that CTL responses are mainly directed against the immunodominant SII peptide epitope. In contrast, splenocytes isolated from mice vaccinated with combination Ova/Tat recognised the immunodominant SII epitope less efficiently, but clearly recognised target cells presenting the subdominant KVV and the cryptic CFD epitopes. Control mice did not recognise any peptide-pulsed EL4 cells.

References for Experiment 1 and the Description

1. Frankel, A. D. & Pabo, C. O. Cellular uptake of the Tat protein from human immunodeficiency virus. *Cell* 55, 1189-1193 (1988).

2. Ensoli, B. *et al.* Release, uptake, and effect of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol.* 67, 277-287 (1993).

3. Fanales-Belasio, E. *et al.* Native HIV-1 Tat protein is selectively taken up by monocyte-derived dendritic cells and induces their maturation, Th-1 cytokine production and antigen presenting function. *J. Immunol.* 168, 197-206 (2002).

4. Cafaro, A. *et al.* Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat. Med.* 5, 643-650 (1999).

5. Cafaro, A. *et al.* Vaccination with DNA containing tat coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P). *Vaccine* 19, 2862-2877 (2001).

6. Pamer, E. & Cresswell, P. Mechanisms of MHC cass I-restricted antigen processing. *Annu. Rev. Immunol.* 16, 323-358 (1998).

7. Rock, K. L. *et al.* Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 78, 761-771 (1994).

8. Rock, K. L. & Goldberg, A. L. Degradation of cell proteins and the generation of MHC class I-presented peptides. *Annu. Rev. Immunol.* 17, 739-779 (1999).

9. Voges, D., Zwickl, P. & Baumeister, W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu. Rev. Biochem.* 68, 1015-1068 (1999).

10. Dick, T. P. *et al.* Contribution of proteasomal β–subunits to the cleavage of peptide substrates analysed with yeast mutants. *J. Biol. Chem.* 273, 25637-25646 (1998).

11. Frisan, T., Levitsky, V., Polack, A. & Masucci, M. Phenotype-dependent differences in proteasome subunit composition and cleavage specificity in B cell lines. *J. Immunol.* 160, 3281-3289 (1998).

12. Morel, S. *et al.* Processing of some antigens by the standard proteasome but not immunoproteasomes results in poor presentation by dendritic cells. *Immunity* 12, 107-117 (2000).

13. Sijts, A. J. A. M. *et al.* Efficient generation of a hepatitis B virus cytotoxic T lymphocyte epitope requires the structural features of immunoproteasomes. *J. Exp. Med.* 191, 503-513 (2000).

14. Gaczynska, M., Rock, K. L. & Goldberg, A. L. γ–Interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature (Lond.)* 365, 264-267 (1993).

15. Cascio, P., Hilton, C., Kisselev, A. F., Rock, K. L. & Goldberg, A. L. 26S proteasomes and immunoproteasomes produce mainly N-extended versions of an antigenic peptide. *EMBO J.* 20, 2357-2366 (2001).

16. Serwold, T., F, G., Kim, J., Jacob, R. & Shastri, N. ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. *Nature (Lond.)* 419, 443-445 (2002).

17. Saric, T. *et al.* An IFN–γ–induced aminopeptidase in the ER, ERAPI, trims precursors to MHC class I-presented peptides. *Nature Immunol.* 3, 1169-1176 (2002).

18. York, I. A. *et al.* The ER aminopeptidase ERAPI enhances or limits antigen-presentation by trimming epitopes to 8-9 residues. *Nature Immunol.* 3, 1177-1184 (2002).

19. Tanaka, K. & Kasahara, M. The MHC class 1 ligand-generating system: roles of immunoproteasomes and the interferon-γ-inducible proteasome activator PA28. *Immunol. Rev.* 163, 161-176 (1998).

20. Niedermann, G. *et al.* Contribution of mediated-mediated proteolysis to the hierarchy of epitopes presented by major histocompatibility complex class I molecules. *Immunity* 2, 289-299 (1995).

21. Mo, A. X. Y., van Lelyveld, S. F. L., Craiu, A. & Rock, K. L. Sequences that flank subdominant and cryptic epitopes influence the proteolytic generation of MHC class I-presented peptides. *J. Immunol.* 164, 4003-4010 (2000).

22. Caputo, A., Sodroski, J. G. & Haseltine, W. A. Constitutive expression of HIV-1 tat protein in human Jurkat T cells using a BK virus vector. *Journal of Acquired Immune Deficiency Syndrome* 3, 372-379 (1990).

23. Gaczynska, K., Rock, K. L., Spies, T. & Goldberg, A. L. Peptidase activities of proteasomes are differentially regulated by the major histocompatibility complex-encoded genes for LMP2 and LMP7. *Proc. Natl. Acad. Sci. USA* 91, 9213-9217 (1994).

24. Groettrup, M. *et al.* The interferon-γ-inducible 11 S regulator (PA28) and the LMP2/LMP7 subunits govern the peptide production by the 20 S proteasome *in vitro*. *J. Biol. Chem.* 270, 23808-23815 (1995).

25. Gaczynska, K., Goldberg, A. L., Tanaka, K., Hendil, K. B. & Rock, K. L. Proteasome subunits X and Y alter peptidase activities in opposite ways to the interferon-γ-induced subunits LMP2 and LMP7. *J. Biol. Chem.* 271, 17275-17280 (1996).

26. Rickinson, A. B. & Moss, D. J. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu. Rev. Immunol.* 15, 405-431 (1997).

27. Gavioli, R. *et al.* Multiple HLA A11-restricted cytotoxic T-lymphocyte epitopes of different immunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. *J. Virol.* 67, 1572-1578 (1993).

28. Lee, S. P. *et al.* HLA A2.1-restricted cytotoxic T cells recognizing a range of Epstein-Barr virus isolates through a defined epitope in latent membrane protein LMP2. *J. Virol.* 67, 7428-7435 (1993).

29. Khanna, R., Burrows, S. R., Nicholls, J. & Poulsen, L. M. Identification of cytotoxic T cell epitopes within Epstein-Barr virus (EBV) oncogene latent membrane protein 1 (LMP1): evidence for HLA A2 supertype-restricted immune recognition of EBV-infected cells by LMP1-specific cytotoxic T lymphocytes. *Eur. J. Immunol.* 28, 451-458 (1998).

30. Lautscham, G. *et al.* Processing of a multiple membrane spanning Epstein-Barr virus protein for CD8+ T cell recognition reveals a dependent-dependent, transporter associated with antigen processing-independent pathway. *J. Exp. Med.* 194, 1053-1068 (2001).

31. Gavioli, R., Vertuani, S. & Masucci, M. G. Proteasome inhibitors reconstitute the presentation of cytotoxic T cell epitopes in Epstein-Barr virus associated tumors. *Int. J. Cancer* 101, 532-538 (2002).

32. Micheletti, F. *et al.* Selective amino acid substitutions of a subdominant Epstein-Barr virus LMP2-derived epitope increase HLA/peptide complex stability and

immunogenicity: implications for immunotherapy of Epstein-Barr virus-associated malignancies. *Eur. J. Immunol.* 29, 2579-2589 (1999).

33. Chen, W., Khilko, S., Fecondo, J., Margulies, D. H. & McCluskey, J. Determinant selection of major histocompatibility complex class I-restricted antigenic peptides is explained by class I-peptide affinity and is strongly influenced by nondominant anchor residues. *J. Exp. Med.* 180, 1471-1483 (1994).

34. Lipford, G. B., Hoffman, M., Wagner, H. & Heeg, K. Primary *in vivo* responses to ovalbumin. *J. Immunol.* 150, 1212-1222 (1993).

35. Zeidler, R. *et al.* Downregulation of TAP1 in B lymphocytes by cellular and Epstein-Barr virus-encoded interleukin-10. *Blood* 90, 2390-2397 (1997).

36. Chatterjee-Kishore, M., van den Akker, F. & Stark, G. R. Adenovirus E1A down-regulates LMP2 transcription by interfering with the binding of Stat1 to IRF1. *J. Biol. Chem.* 275, 20406-20411 (2000).

37. Turnell, A. S. *et al.* Regulation of the 26S proteasome by adenovirus E1A. *EMBO J.* 19, 4759-4773 (2000).

38. André, P. *et al.* An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses. *Proc. Natl. Acad. Sci. USA* 95, 13120-13124 (1998).

39. Berezutskaya, E. & Bagchi, S. The human papillomavirus E7 oncoprotein functionally interacts with the S4 subunit of the 26S proteasome. *J. Biol. Chem.* 272, 30135-30140 (1997).

40. Ehrlich, R. Modulation of antigen processing and presentation by persistent virus infections and in tumors. *Hum. Immunol.* 54, 104-116 (1997).

41. Schmidtke, G. *et al.* How an inhibitor of the HIV-I protease modulates proteasome activity. *J. Biol. Chem.* 274, 35734-35740 (1999).

42. Hu, Z. *et al.* Hepatitis B virus X protein is both substrate and a potential inhibitor of the proteasome complex. *J. Virol.* 73, 7231-7240 (1999).

43. Wing, S. S. & Goldberg, A. L. Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. *American Journal of Physiology* 264, 668-676 (1993).

44. Gavioli, R., Frisan, T., Vertuani, S., Bornkamm, G. W. & Masucci, M. G. c-Myc overexpression activates alternative pathways for intracellular proteolysis in lymphoma cells. *Nat. Cell Biol.* 3, 283-288 (2001).

45. Cerundolo, V., Kelly, A., Elliot, T., Trowsdale, J. & Townsend, A. Genes encoded in the major histocompatibility complex affecting the generation of peptides for TAP transport. *Eur. J. Immunol.* 25, 554-562 (1995).

46. Sewell, A. K. *et al.* IFN-γ exposes a cryptic cytotoxic T lymphocyte epitope in HIV-1 reverse transcriptase. *J. Immunol.* 162, 7075-7079 (1999).

47. van Hall, T. *et al.* Differential influence on cytotoxic T lymphocyte epitope presentation by controlled expression of either proteasome immunosubunits or PA28. *J. Exp. Med.* 192, 483-494 (2000).

48. Sijts, A. J. A. M. *et al.* MHC class I antigen processing of an adenovirus CTL epitope is linked to the levels of immunoproteasomes in infected cells. *J. Immunol.* 164, 4500-4506 (2000).

49. Schmidtke, G. *et al.* Inactivation of a defined active site in the mouse 20S proteasome complex enhances major histocompatibility complex class I antigen presentation of a murine cytomegalovirus protein. *J. Exp. Med.* 187, 1641-1646 (1998).

50. Chen, W., Norbury, C. C., Cho, Y., Yewdell, J. W. & Bennink, J. R. Immunoproteasomes shape immunodominance hierarchies of antiviral CD8+ T cells at the levels of T cell repertoire and presentation of viral antigens. *J. Exp. Med.* 193, 1319-1326 (2001).

51. Groll, M. *et al.* The catalytic sites of 20S proteasomes and their role in subunit maturation: a mutational and crystallographic study. *Proc. Natl. Acad. Sci. USA* 96, 10976-10983 (1999).

52. Feltkamp, M. C. W. *et al.* Cytotoxic T lymphocytes raised against a subdominant epitope offered as a synthetic peptide eradicate human papilloma virus type 16-induced tumors. *Eur. J. Immunol.* 25, 2638-2642 (1995).

53. Rubartelli, A., Poggi, A., Sitia, R. & Zocchi, M. R. HIV-I Tat: a polypeptide for all seasons. *Immunol. Today* 19, 543-545 (1998).

54. Miller, A. D. *et al.* Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J. Virol.* 65, 2220-2224 (1991).

55. Morgenstern, J. P. & Land, H. Advanced mammalian gene transfer: high titre retroviral vectors with nultiple drug selection markers and a complementary helper-free packaging cell line. *Nucleic Acids Res.* 18, 3587-3596 (1990).

56. Graham, F. L. & van der Eb, A. J. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52, 456-467 (1973).

57. Micheletti, F. *et al.* Supra-agonist peptides enhance the reactivation of memory cytotoxic T lymphocyte responses. *J. Immunol.* 165, 4264-4271 (2000).

Example 2

HIV-1 Tat-mutant and an HIV Tat-derived Peptides Modulate Proteasome Composition and Enzymatic Activity

Materials and Methods

Cells

Jurkat T cells expressing Tat or mutated Tat (referred to as Tat22, wherein a Cys at position 22 is mutated) have previously been described (1). Cells were cultured in a medium supplemented with 800 μg/ml neomycin (Sigma, St. Louise, MI).

HIV-1 Tat protein

HIV-1 Tat from the human T lymphotropic virus type IIIB isolate (BH10 clone) was expressed in *E. Coli* and purified by heparin-affinity chromatography and HPLC as previously described (2). The lyophilised Tat protein was stored at -80°C to prevent oxidation, reconstituted in degassed buffer before use, and handled as described (3). Different lots of Tat were used with reproducible results, and, in all cases, endotoxin concentration was undetectable (detection threshold: 0.05 EU/μg).

Purification of proteasomes

Cells were lysed with glass beads as previously described (4). Supernatants were ultracentrifuged for 1 h at 100,000 *g* and loaded into an affinity column containing a matrix derivatised with the MCP21 mAb specific for the α2 subunit of the proteasome (Affinity, Exeter, UK). Proteasomes were eluted with 25 mM Tris-HCl pH 7.5 containing 2 M NaCl, and 0.5 ml fractions were collected. Homogeneity of the eluted material was confirmed by analysis of an aliquot by SDS 12% PAGE and Coomassie blue staining of the gel. Fractions containing proteasomes were combined and dialysed against 25 mM Tris-HCl pH 7.5. Protein concentration was determined using the BCA method (Pierce Chemical, Rockford, IL).

Western Blot assay

Equal amounts of proteins, or equal amounts of purified proteasomes, were loaded on a 12% SDS-PAGE and electro-blotted onto Protran nitrocellulose membranes

(Schleicher & Schuell, Keene, Hampshire, USA). Blots were probed with Abs specific for α2, LMP2 LMP7, MECL1, and PA28α subunits (Affinity), and developed by enhanced chemi-luminescence (ECL, Amersham Pharmacia Biotech, Uppsala, SW).

Enzymatic assays

The chymotrypsin-like, trypsin-like and post-acidic activities of purified proteasomes were tested using the fluorogenic substrates Suc-LLVY-AMC, Boc-LRR-AMC and Ac-YVAD-AMC, respectively, as previously described (4). Fluorescence was determined by a fluorimeter (Spectrafluor plus, Tecan, Salzburg, Austria). Proteasome activity is expressed as arbitrary fluorescence units.

Synthetic peptides

Peptides were synthesized by the solid phase method and purified by HPLC to >98% purity, as previously described (5). Structure verification was performed by elemental and amino acid analysis and mass spectrometry. Peptides were dissolved in DMSO at 10^{-2} M, kept at -20°C, and diluted in PBS before use.

Results and Discussion

Endogenously expressed Tat or exogenous native Tat protein modulate proteasome composition and activity in Jurkat cells

We have shown in Example 1 that the HIV-1 Tat modifies the catalytic subunit composition and activity of immunoproteasomes in lymphoblastoid cell lines which either express Tat or have been treated with exogenous biological active Tat protein. Similarly, the endogenous expression of Tat in Jurkat cells induces down-regulation of LMP2 and up-regulation of LMP7 and MECL1 (see Example 1 and and Fig. 10).

To assay whether the exogenous Tat protein modulates the expression of the catalytic subunits of proteasomes in Jurkat cells, we evaluated the expression of proteasomes from Jurkat cells cultured for 12 (Fig. 11A) and 24 hrs (Fig. 11B) in the absence or presence of increasing concentrations of the native Tat protein. After treatment, expression of LMP2 subunit from purified proteasomes was evaluated as a marker of Tat-induced proteasomal modification. As shown in Fig.11, maximal down-

modulation of the LMP2 subunit was observed after 24 h of treatment with 0.1-1 μg/ml of Tat.

These results demonstrate that both endogenously expressed Tat and exogenous native Tat protein modify the subunit composition of immunoproteasomes in Jurkat cells.

To investigate whether the differences in subunit composition correlated with differences in enzymatic activity, we analysed the cleavage specificity of equal amounts of proteasomes purified from Jurkat cells treated for 24 h with 1μg/ml Tat protein or from control cells. Chymotryptic-like, tryptic-like, and post-acidic activities were all augmented in proteasomes purified from Jurkat cells treated with Tat, as compared to control cells (Fig. 12).

Tat does not require Cysteine 22 to modulate proteasome composition and activity

In the next set of experiments we evaluated the effect of Tat mutants stably expressed in Jurkat cells. Cysteines at position 22 and/or 37 were substituted with glycine and serine, respectively, to obtain three mutant Tat molecules (Tat22, Tat37 and Tat22/37). Tat22 and Tat22/37 mutants, in contrast to wild-type Tat, have no effect on the transactivation of the HIV-1 LTR, and do not induce reactivation of latent infection.

The level of expression of proteasomes was then analysed in Tat-expressing cells and compared to cells expressing Tat mutants. No difference in proteasome expression was detected in these cells by the use of a monoclonal antibody specific for the α2-subunit (Fig. 13). In contrast, a marked down-regulation of the LMP2 subunit was observed in proteasomes purified from cells expressing Tat mutants, as previously demonstrated for cells expressing wild-type Tat.

The Tat-derived 47-86 peptide is sufficient to down-modulate the LMP2 subunit

To identify the region of Tat responsible for the modulation of the catalytic subunits of immuno-proteasomes, we tested the effect of peptides 1-38, 21-58 and 47-86 covering the wild-type sequence of Tat. Jurkat cells were treated for 24 h with 0,1 μg/ml of Tat-derived peptides and, after treatment, total cell lysates were assayed for proteasome expression by western-blot. As shown in Fig.14, Tat protein and peptides 47-86 induced down-regulation of LMP2, while the other peptides showed no discernable effect.

Conclusions

We have shown here that a mutated form of the HIV-1 Tat protein, carrying a glycine instead of cysteine 22 (Tat22), like wild-type Tat, modifies the subunit composition of proteasomes. In addition, we demonstrated that peptide 47-86, derived from the wild-type Tat, protein is sufficient to down-modulate LMP2 subunit. We have recently shown that LMP2 down-regulation by wild-type Tat results in a different generation of CTL epitopes in virally infected cells (6). Furthermore, we have produced evidence suggesting that Tat modifies *in vivo* CTL responses against heterologous antigens favouring the generation of subdominant CTL epitopes (unpublished results). Therefore, mutated forms of Tat or Tat-derived peptide may represent an important alternative to the use of wild-type Tat in vaccination strategies aimed at increasing epitope-specific T cell responses directed to heterologous antigens.

References for Example 2

1. Caputo, A., J. G. Sodroski, and W. A. Haseltine. 1990. Constitutive expression of HIV-1 tat protein in human Jurkat T cells using a BK virus vector. *Journal of Acquired Immune Deficiency Syndrome 3:372.*

2. Fanales-Belasio, E., S. Moretti, F. Nappi, G. Barillari, F. Micheletti, A. Cafaro, and B. Ensoli. 2002. Native HIV-1 Tat protein is selectively taken up by monocyte-derived dendritic cells and induces their maturation, Th-1 cytokine production and antigen presenting function. *J. Immunol. 168:197.*

3. Ensoli, B., L. Buonaguro, G. Barillari, V. Fiorelli, R. Gendelman, R. A. Morgan, P. Wingfield, and R. C. Gallo. 1993. Release, uptake, and effect of

extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol. 67:277.*

4. Gavioli, R., T. Frisan, S. Vertuani, G. W. Bornkamm, and M. G. Masucci. 2001. c-Myc overexpression activates alternative pathways for intracellular proteolysis in lymphoma cells. *Nat. Cell Biol. 3:283.*

5. Micheletti, F., A. Canella, S. Vertuani, M. Marastoni, L. Tosi, S. Volinia, S. Traniello, and R. Gavioli. 2000. Supra-agonist peptides enhance the reactivation of memory cytotoxic T lymphocyte responses. *J. Immunol. 165:4264.*

6. Gavioli, R., E. Gallerani, C. Fortini, M. Fabris, A. Bottoni, A. Canella, A. Bonaccorsi, M. Marastoni, F. Micheletti, A. Cafaro, P. Rimessi, A. Caputo, and B. Ensoli. 2004. HIV-1 Tat protein modulates cytotoxic T cell epitopes by modifying proteasome composition and enzymatic activity. *J. Immunol. 173:3838.*

Example 3

HIV-1 Tat Protein increases cytotoxic T cell epitopes recognized within heterologous HIV-1 structural Gag and Env antigens

Introduction

We shown above that the HIV-1 Tat protein modulates in vitro CTL epitope hierarchy by modifying the catalytic subunit composition of immunoproteasome. In particular, by up-regulating LMP7 and MECL1 subunits and by down-modulating the LMP2 subunit, both intracellularly expressed or exogenous native Tat protein increase the major proteolytic activities of the proteasome resulting in a more efficient generation and presentation of subdominant CTL epitopes. Since the amount of MHC-I/epitope complexes is crucial in determining the presence and the strength of epitope-specific CTL responses and to verify the biological relevance of these findings for vaccination strategies, we evaluated epitope-specific CTL responses against ovalbumin in mice vaccinated with both Tat and ovalbumin.

We found that Tat slightly decreases CTL responses directed to the immunodominant epitope while induces those directed to subdominant and cryptic T-cell epitopes that were not present in mice vaccinated with ovalbumin alone. Due to these effects we exploited the effect of Tat on T cell responses against structural HIV gene products. We found that Tat increases the number of CTL epitopes within Gag and Env antigens. Thus, Tat may represent a new tool to induce new epitope-specific CTL responses against HIV antigens and could be used as co-antigen for the development of new vaccination strategies against AIDS.

Materials and Methods

HIV-1 proteins.

HIV-1 Tat and the mutant Tatcys22 (C→G) from the human T lymphotropic virus type IIIB isolate (BH10 clone) was expressed in *E. Coli* and purified by heparin-affinity chromatography and HPLC as described previously (2). The Tat proteins were stored lyophilised at -80°C to prevent oxidation, reconstituted in degassed buffer before use, and handled as described (4). Different lots of Tat were used with

reproducible results, and in all cases endotoxin concentration was undetectable (detection threshold: 0.05 EU/μg). HIV-1 GagSF2 and HIV-1 EnvSF2 proteins were obtained from Chiron and NIH AIDS Reagent Program respectively (HIV-1 gp120 SF162; # 7363). The Gag sequence (HIVSF2 p55) is given in SEQ ID NO 266. The Env sequence (HIV-1 SF162 gp120) is given in SEQ ID NOS 267 (without linker) and 288 (with linker). The entire HIV-1 Env gp160 SF162 sequence is given in SEQ ID NO. 287.

Synthetic peptides.

Peptides were synthesized by solid phase method and purified by HPLC to >98% purity, as previously described (5). Structure verification was performed by elemental and amino acid analysis and mass spectrometry.

Gag and Env peptides, 15 amino acid long and overlapping by 10 to 11 amino acids, spanning the entire Gag (HIV-1 consensus subtype B Gag complete set, # 8117) and Env sequences (SHIV SF162P3 env set; # 7619 and HIV-1 consensus subtype B Env complete set, # 9840), were provided by NIH AIDS Reagent Program. Peptides were dissolved in DMSO at 10^{-3} M, kept at -20°C, and diluted in PBS before use. The Gag peptides are listed in Table 1 and the Env peptides are listed in Table 2. The amino acid number relative to the full sequences are given, together with the appropriate SEQ ID NO from the Sequence Listing.

Table 1: Gag peptides

Reference Name	Sequence	Amino Acid Position	SEQ ID NO.
Gag 1	MGARASVLSGGELDR	1-15	1
Gag 2	ASVLSGGELDRWEKI	5-19	2
Gag 3	SGGELDRWEKIRLRP	9-23	3
Gag 4	LDRWEKIRLRPGGKK	13-27	4
Gag 5	EKIRLRPGGKKKYKL	17-31	5
Gag 6	LRPGGKKKYKLKHIV	21-35	6
Gag 7	GKKKYKLKHIVWASR	25-39	7

Gag 8	YKLKHIVWASRELER	29-43	8
Gag 9	HIVWASRELERFAVN	33-47	9
Gag 10	ASRELERFAVNPGLL	37-51	10
Gag 11	LERFAVNPGLLETSE	41-55	11
Gag 12	AVNPGLLETSEGCRQ	45-59	12
Gag 13	GLLETSEGCRQILGQ	49-63	13
Gag 14	TSEGCRQILGQLQPS	53-67	14
Gag 15	CRQILGQLQPSLQTG	57-71	15
Gag 16	LGQLQPSLQTGSEEL	61-75	16
Gag 17	QPSLQTGSEELRSLY	65-79	17
Gag 18	QTGSEELRSLYNTVA	69-83	18
Gag 19	EELRSLYNTVATLYC	73-87	19
Gag 20	SLYNTVATLYCVHQR	77-91	20
Gag 21	TVATLYCVHQRIEVK	81-95	21
Gag 22	LYCVHQRIEVKDTKE	85-99	22
Gag 23	HQRIEVKDTKEALEK	89-103	23
Gag 24	EVKDTKEALEKIEEE	93-107	24
Gag 25	TKEALEKIEEEQNKS	97-111	25
Gag 26	LEKIEEEQNKSKKKA	101-115	26
Gag 27	EEEQNKSKKKAQQAA	105-119	27
Gag 28	NKSKKKAQQAAADTG	109-123	28
Gag 29	KKAQQAAADTGNSSQ	113-127	29
Gag 30	QAAADTGNSSQVSQN	117-131	30
Gag 31	DTGNSSQVSQNYPIV	121-135	31
Gag 32	SSQVSQNYPIVQNLQ	125-139	32
Gag 33	SQNYPIVQNLQGQMV	129-143	33
Gag 34	PIVQNLQGQMVHQAI	133-147	34
Gag 35	NLQGQMVHQAISPRT	137-151	35
Gag 36	QMVHQAISPRTLNAW	141-155	36
Gag 37	QAISPRTLNAWVKVV	145-159	37
Gag 38	PRTLNAWVKVVEEKA	149-163	38
Gag 39	NAWVKVVEEKAFSPE	153-167	39

Gag 40	KVVEEKAFSPEVIPM	157-171	40
Gag 41	EKAFSPEVIPMFSAL	161-175	41
Gag 42	SPEVIPMFSALSEGA	165-179	42
Gag 43	IPMFSALSEGATPQD	169-183	43
Gag 44	SALSEGATPQDLNTM	173-187	44
Gag 45	EGATPQDLNTMLNTV	177-191	45
Gag 46	PQDLNTMLNTVGGHQ	181-195	46
Gag 47	NTMLNTVGGHQAAMQ	185-199	47
Gag 48	NTVGGHQAAMQMLKE	189-203	48
Gag 49	GHQAAMQMLKETINE	193-207	49
Gag 50	AMQMLKETINEEAAE	197-211	50
Gag 51	LKETINEEAAEWDRL	201-215	51
Gag 52	INEEAAEWDRLHPVH	205-219	52
Gag 53	AAEWDRLHPVHAGPI	209-223	53
Gag 54	DRLHPVHAGPIAPGQ	213-227	54
Gag 55	PVHAGPIAPGQMREP	217-231	55
Gag 56	GPIAPGQMREPRGSD	221-235	56
Gag 57	PGQMREPRGSDIAGT	225-239	57
Gag 58	REPRGSDIAGTTSTL	229-243	58
Gag 59	GSDIAGTTSTLQEQI	233-247	59
Gag 60	AGTTSTLQEQIGWMT	237-251	60
Gag 61	STLQEQIGWMTNNPP	241-255	61
Gag 62	EQIGWMTNNPPIPVG	245-259	62
Gag 63	WMTNNPPIPVGEIYK	249-263	63
Gag 64	NPPIPVGEIYKRWII	253-267	64
Gag 65	PVGEIYKRWIILGLN	257-271	65
Gag 66	IYKRWIILGLNKIVR	261-275	66
Gag 67	WIILGLNKIVRMYSP	265-279	67
Gag 68	GLNKIVRMYSPTSIL	269-283	68
Gag 69	IVRMYSPTSILDIRQ	273-287	69
Gag 70	YSPTSILDIRQGPKE	277-291	70
Gag 71	SILDIRQGPKEPFRD	281-295	71

Gag 72	IRQGPKEPFRDYVDR	285-299	72
Gag 73	PKEPFRDYVDRFYKT	289-303	73
Gag 74	FRDYVDRFYKTLRAE	293-307	74
Gag 75	VDRFYKTLRAEQASQ	297-311	75
Gag 76	YKTLRAEQASQEVKN	301-315	76
Gag 77	RAEQASQEVKNWMTE	305-319	77
Gag 78	ASQEVKNWMTETLLV	309-323	78
Gag 79	VKNWMTETLLVQNAN	313-327	79
Gag 80	MTETLLVQNANPDCK	317-331	80
Gag 81	LLVQNANPDCKTILK	321-335	81
Gag 82	NANPDCKTILKALGP	325-339	82
Gag 83	DCKTILKALGPAATL	329-343	83
Gag 84	ILKALGPAATLEEMM	333-347	84
Gag 85	LGPAATLEEMMTACQ	337-351	85
Gag 86	ATLEEMMTACQGVGG	341-355	86
Gag 87	EMMTACQGVGGPGHK	345-359	87
Gag 88	ACQGVGGPGHKARVL	349-363	88
Gag 89	VGGPGHKARVLAEAM	353-367	89
Gag 90	GHKARVLAEAMSQVT	357-371	90
Gag 91	RVLAEAMSQVTNSAT	361-375	91
Gag 92	EAMSQVTNSATIMMQ	365-379	92
Gag 93	QVTNSATIMMQRGNF	369-383	93
Gag 94	SATIMMQRGNFRNQR	373-387	94
Gag 95	MMQRGNFRNQRKTVK	377-391	95
Gag 96	GNFRNQRKTVKCFNC	381-395	96
Gag 97	NQRKTVKCFNCGKEG	385-399	97
Gag 98	TVKCFNCGKEGHIAK	389-403	98
Gag 99	FNCGKEGHIAKNCRA	393-407	99
Gag 100	KEGHIAKNCRAPRKK	397-411	100
Gag 101	IAKNCRAPRKKGCWK	401-415	101
Gag 102	CRAPRKKGCWKCGKE	405-419	102
Gag 103	RKKGCWKCGKEGHQM	409-423	103

Gag 104	CWKCGKEGHQMKDCT	413-427	104
Gag 105	GKEGHQMKDCTERQA	417-431	105
Gag 106	HQMKDCTERQANFLG	421-435	106
Gag 107	DCTERQANFLGKIWP	425-439	107
Gag 108	RQANFLGKIWPSHKG	429-443	108
Gag 109	FLGKIWPSHKGRPGN	433-447	109
Gag 110	IWPSHKGRPGNFLQS	437-451	110
Gag 111	HKGRPGNFLQSRPEP	441-455	111
Gag 112	PGNFLQSRPEPTAPP	445-459	112
Gag 113	LQSRPEPTAPPEESF	449-463	113
Gag 114	PEPTAPPEESFRFGE	453-467	114
Gag 115	APPEESFRFGEETTT	457-471	115
Gag 116	ESFRFGEETTTPSQK	461-475	116
Gag 117	FGEETTTPSQKQEPI	465-479	117
Gag 118	TTTPSQKQEPIDKEL	469-483	118
Gag 119	SQKQEPIDKELYPLA	473-487	119
Gag 120	EPIDKELYPLASLRS	477-491	120
Gag 121	KELYPLASLRSLFGN	481-495	121
Gag 122	PLASLRSLFGNDPSS	485-499	122
Gag 123	LRSLFGNDPSSQ	489-500	123

Table 2 : Env peptides

Reference Name	Sequence	Amino Acid Position	SEQ ID NO.
Env 1	MRVKGIRKNYQHLWR	aa 1-15	124
Env 2	GIRKNYQHLWRGGTL	aa 5-19	125
Env 3	NYQHLWRGGTLLLGM	aa 9-23	126
Env 4	LWRGGTLLLGMLMIC	aa 13-27	127
Env 5	GTLLLGMLMICSAVE	aa 17-31	128
Env 6	LGMLMICSAVEKLWV	aa 21-35	129

Env 7	MICSAVEKLWVTVYY	aa 25-39	130
Env 8	AVEKLWVTVYYGVPA	aa 29-43	131
Env 9	LWVTVYYGVPAWKEA	aa 33-47	132
Env 10	VYYGVPAWKEATTTL	aa 37-51	133
Env 11	VPAWKEATTTLFCAS	aa 41-55	134
Env 12	KEATTTLFCASDAKA	aa 45-59	135
Env 13	TTLFCASDAKAYDTE	aa 49-63	136
Env 14	CASDAKAYDTEVHNV	aa 53-67	137
Env 15	AKAYDTEVHNVWATH	aa 57-71	138
Env 16	DTEVHNVWATHACVP	aa 61-75	139
Env 17	HNVWATHACVPTDPN	aa 65-79	140
Env 18	ATHACVPTDPNPQEI	aa 69-83	141
Env 19	CVPTDPNPQEIVLEN	aa 73-87	142
Env 20	DPNPQEIVLENVTEN	aa 77-91	143
Env 21	PQEIVLENVTENFNM	aa 80-94	144
Env 22	VLENVTENFNMWKNN	aa 84-98	145
Env 23	VTENFNMWKNNMVEQ	aa 88-102	146
Env 24	FNMWKNNMVEQMHED	aa 92-106	147
Env 25	KNNMVEQMHEDIISL	aa 96-110	148
Env 26	VEQMHEDIISLWDQS	aa 100-114	149
Env 27	HEDIISLWDQSLEPC	aa 104-118	150
Env 28	ISLWDQSLEPCVKLT	aa 108-122	151
Env 29	DQSLEPCVKLTPLCV	aa 112-126	152
Env 30	EPCVKLTPLCVTLHC	aa 116-130	153
Env 31	KLTPLCVTLHCTNLE	aa 120-134	154
Env 32	LCVTLHCTNLENATN	aa 124-138	155
Env 33	LHCTNLENATNTTSS	aa 128-142	156
Env 34	NLENATNTTSSNWKE	aa 132-146	157
Env 35	ATNTTSSNWKEMNRG	aa 136-150	158
Env 36	TSSNWKEMNRGEIKN	aa 140-154	159
Env 37	WKEMNRGEIKNCSFN	aa 144-158	160
Env 38	NRGEIKNCSFNVTTS	aa 148-162	161

Env 39	IKNCSFNVTTSIGNK	aa 152-166	162
Env 40	SFNVTTSIGNKMQKE	aa 156-170	163
Env 41	TTSIGNKMQKEYALF	aa 160-174	164
Env 42	GNKMQKEYALFYRLD	aa 164-178	165
Env 43	MQKEYALFYRLDVVP	aa 167-181	166
Env 44	YALFYRLDVVPIDND	aa 171-185	167
Env 45	YRLDVVPIDNDNTSY	aa 175-189	168
Env 46	VVPIDNDNTSYNLIN	aa 179-193	169
Env 47	DNDNTSYNLINCNTS	aa 183-197	170
Env 48	TSYNLINCNTSVITQ	aa 187-201	171
Env 49	LINCNTSVITQACPK	aa 191-205	172
Env 50	NTSVITQACPKVSFE	aa 195-209	173
Env 51	ITQACPKVSFEPIPI	aa 199-213	174
Env 52	CPKVSFEPIPIHYCA	aa 203-217	175
Env 53	SFEPIPIHYCAPAGF	aa 207-221	176
Env 54	IPIHYCAPAGFAILK	aa 211-225	177
Env 55	YCAPAGFAILKCNDK	aa 215-229	178
Env 56	AGFAILKCNDKKFNG	aa 219-233	179
Env 57	ILKCNDKKFNGSGPC	aa 223-237	180
Env 58	NDKKFNGSGPCINVS	aa 227-241	181
Env 59	FNGSGPCINVSTVQC	aa 231-245	182
Env 60	GPCINVSTVQCTHGI	aa 235-249	183
Env 61	NVSTVQCTHGIRPVV	aa 239-253	184
Env 62	VQCTHGIRPVVSTQL	aa 243-257	185
Env 63	HGIRPVVSTQLLLNG	aa 247-261	186
Env 64	PVVSTQLLLNGSLAE	aa 251-265	187
Env 65	TQLLLNGSLAEEGVV	aa 255-269	188
Env 66	LNGSLAEEGVVIRSE	aa 259-273	189
Env 67	LAEEGVVIRSENFTD	aa 263-277	190
Env 68	GVVIRSENFTDNVKT	aa 267-281	191
Env 69	RSENFTDNVKTIIVQ	aa 271-285	192
Env 70	FTDNVKTIIVQLKES	aa 275-289	193

Env 71	VKTIIVQLKESVEIN	aa 279-293	194
Env 72	IVQLKESVEINCTRP	aa 283-297	195
Env 73	KESVEINCTRPNNNT	aa 287-301	196
Env 74	EINCTRPNNNTRKSI	aa 291-305	197
Env 75	TRPNNNTRKSIPIGP	aa 295-309	198
Env 76	NNTRKSIPIGPGKAF	aa 299-313	199
Env 77	KSIPIGPGKAFYATG	aa 303-317	200
Env 78	IGPGKAFYATGDIIG	aa 307-321	201
Env 79	KAFYATGDIIGDIRQ	aa 311-325	202
Env 80	ATGDIIGDIRQAHCN	aa 315-329	203
Env 81	IIGDIRQAHCNISGE	aa 319-333	204
Env 82	IRQAHCNISGEKWNN	aa 323-337	205
Env 83	HCNISGEKWNNTLKQ	aa 327-341	206
Env 84	SGEKWNNTLKQIVTK	aa 331-345	207
Env 85	WNNTLKQIVTKLQAQ	aa 335-349	208
Env 86	LKQIVTKLQAQFENK	aa 339-353	209
Env 87	VTKLQAQFENKTIVF	aa 343-357	210
Env 88	LQAQFENKTIVFKQS	aa 346-360	211
Env 89	FENKTIVFKQSSGGD	aa 350-364	212
Env 90	TIVFKQSSGGDPEIV	aa 354-368	213
Env 91	KQSSGGDPEIVMHSF	aa 358-372	214
Env 92	GGDPEIVMHSFNCGG	aa 362-376	215
Env 93	EIVMHSFNCGGEFFY	aa 366-380	216
Env 94	HSFNCGGEFFYCNST	aa 370-384	217
Env 95	CGGEFFYCNSTQLFN	aa 374-388	218
Env 96	FFYCNSTQLFNSTWN	aa 378-392	219
Env 97	NSTQLFNSTWNNTIG	aa 382-396	220
Env 98	LFNSTWNNTIGPNNT	aa 386-400	221
Env 99	TWNNTIGPNNTNGTI	aa 390-404	222
Env 100	TIGPNNTNGTITLPC	aa 394-408	223
Env 101	NNTNGTITLPCRIKQ	aa 398-412	224
Env 102	GTITLPCRIKQIINR	aa 402-416	225

Env 103	LPCRIKQIINRWQEV	aa 406-420	226
Env 104	IKQIINRWQEVGKAM	aa 410-424	227
Env 105	INRWQEVGKAMYAPP	aa 414-128	228
Env 106	WQEVGKAMYAPPIRG	aa 417-431	229
Env 107	GKAMYAPPIRGQIRC	aa 421-435	230
Env 108	YAPPIRGQIRCSSNI	aa 425-439	231
Env 109	IRGQIRCSSNITGLL	aa 429-443	232
Env 110	IRCSSNITGLLLTRD	aa 433-447	233
Env 111	SNITGLLLTRDGGRE	aa 437-451	234
Env 112	GLLLTRDGGREVGNT	aa 441-455	235
Env 113	TRDGGREVGNTTEIF	aa 445-459	236
Env 114	GREVGNTTEIFRPGG	aa 449-463	237
Env 115	GNTTEIFRPGGGDMR	aa 453-467	238
Env 116	EIFRPGGGDMRDNWR	aa 457-471	239
Env 117	PGGGDMRDNWRSELY	aa 461-475	240
Env 118	DMRDNWRSELYKYKV	aa 465-479	241
Env 119	NWRSELYKYKVVKIE	aa 469-483	242
Env 120	ELYKYKVVKIEPLGV	aa 473-487	243
Env 121	YKVVKIEPLGVAPTK	aa 477-491	244
Env 122	KIEPLGVAPTKAKRR	aa 481-495	245
Env 123	LGVAPTKAKRRVVQR	aa 485-499	246
Env 124	PTKAKRRVVQREKRA	aa 489-503	247
Env 125	KRRVVQREKRAVTLG	aa 493-507	248
Env 126	VQREKRAVTLGAVFL	aa 497-511	249
Env 127	KRAVTLGAVFLGFLG	aa 501-515	250
Env 128	TLGAVFLGFLGAAGS	aa 505-519	251
Env 129	VFLGFLGAAGSTMGA	aa 509-523	252
Env 130	FLGAAGSTMGAASLT	aa 513-527	253
Env 131	AGSTMGAASLTLTVQ	aa 517-531	254
Env 132	MGAASLTLTVQARQL	aa 521-535	255
Env 133	SLTLTVQARQLLSGI	aa 525-539	256
Env 8771[c]	LWVTVYYGVPVWKEA	aa 33-47	257

Env 8772 [c]	VYYGVPVWKEATTTL	aa 37-51	258
Env 8773 [c]	VPVWKEATTTLFCAS	aa 41-55	259
Env 8789 [c]	EDIISLWDQSLKPCV	aa 104-118	260
Env 8790 [c]	SLWDQSLKPCVKLTP	aa 108-122	261
Env 8791 [c]	QSLKPCVKLTPLCVT	aa 112-126	262
Env 8805 [c]	QKEYALFYKLDVVPI	aa 168-182	263
Env 8806 [c]	ALFYKLDVVPIDNDN	aa 172-186	264
Env 8822 [c]	GPCTNVSTVQCTHGI	aa 235-249	265

Mice immunization.

C57BL/6 mice (H-2^b) (Harlan Nossan, Udine, I) were immunized subcutaneously, in a single site in the back, with 25 μg of ovalbumin (Sigma) alone or in combination with native monomeric biologically active Tat protein (5 and 10 μg, respectively) in Freund's adjuvant (CFA for the first injection, and IFA for subsequent injections). BALB/c mice (H-2^d) (Harlan, Udine, Italy) were immunized subcutaneously, in a single site in the back, with 5 μg of HIV-1 Gag or Env proteins alone or in combination with 5 μg of native monomeric biologically active Tat protein or with the mutant Tatcys22 protein in Freund's adjuvant or in Alum. Each group was composed of 5 animals. Immunogens were given subcutaneously in 100 μl, at days 1, 14 and 28. Mice were sacrificed 10 days after the last boost (day 38). During the course of the experiments, animals were controlled twice a week at the site of injection and for their general conditions (such as liveliness, food intake, vitality, weight, motility, sheen of hair). No signs of local nor systemic adverse reactions were ever observed in mice receiving the immunogens as compared to control or untreated mice. Animal use was according to European and institutional guidelines.

Splenocytes purification.

Splenocytes were purified from spleens squeezed on filters (Cell Strainer, 70 μm, Nylon, Becton Dickinson). Spleens of each experimental group were pooled. Following red blood cell lysis with of 154 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA (5 ml/spleen) for 4 minutes at room temperature, cells were diluted with RPMI 1640 containing 3% FBS (Hyclone), spun for 10 minutes at 1200 rpm, resuspended in

RPMI 1640 containing 10% FBS and used immediately for the analysis of antigen-specific cellular immune responses (fresh). Cellular responses were also measured after in vitro re-stimulation. Therefore, cells (3-5 x 10^6/ml) were stimulated in 14 ml with the Env 10-mer (3 μg/ml), or with pools of Env peptides (15-mers) (10^{-6} M) for 5 days before Elispot analysis.

Cytotoxicity assay.

Target cells were labelled with $Na_2{}^{51}CrO_4$ for 90 min at 37° C. Cytotoxicity test were routinely run at different effector : target ratios in triplicate. Percent specific lysis was calculated as 100 x (cpm sample - cpm medium)/(cpm Triton X-100 - cpm medium) (6). Spontaneous release was always less than 20%.

Elispot assays.

Elispot (IFN-γ) was carried out using a commercially available kit provided by Becton Dickinson (murine IFNgamma ELISPOT Set; # 551083), according to manufacturer's instructions. Briefly, nitrocellulose 96-well plates were coated with 5 μg/ml of anti-IFN-γ overnight at 4°C. The following day, plates were washed 4 times with PBS and blocked with RPMI 1640 supplemented with 10% foetal bovine serum for 2 hours at 37°C. Splenocytes (2.5 and 5 x 10^5/200 μl for assays on fresh cells, and 5 x 10^4/200 μl for assays on cells in vitro re-stimulated) were added to the wells (duplicate wells) and incubated with peptides (10^{-6} M) for 16 hours at 37°C. Controls were represented by cells incubated with Concanavaline A (Sigma; 5 μg/ml) (positive control) or with medium alone (negative control). The spots were read using an Elispot reader (Elivis, Germany). The results are expressed as neat number of spot forming units (SFU)/10^6 cells: [mean number SFU of peptide treated wells minus mean number SFU of the negative control].

Results and Discussion

In vivo modulation of epitope-specific CTL responses against ovalbumin by the HIV-1 Tat protein.

We demonstrated in Example 1 that, by altering the antigen processing machinery, Tat influences the number of MHC class I-epitope complexes at the cell surface of

antigen presenting cells, thereby modulating CTL responses directed against immunodominant and subdominant epitopes within heterologous antigens. To determine the relevance of these *in vitro* findings, the effect of Tat on the induction of epitope-specific CTL responses was investigated *in vivo*.

To this end, K^b-restricted CTL responses to ovalbumin (Ova) that are directed to the immunodominant SIINFEKL (SII) epitope (SEQ ID NO 268) and potentially to the subdominant KVVRFDKL (KVV) (SEQ ID NO 269) and the cryptic CFDVFKEL (CFD) (SEQ ID NO 270) epitopes (7, 8) were used as model systems. In fact, it has been shown that KVV- and CFD-specific CTLs are not found upon immunization of C57BL/6 mice with Ova and that the lack of these responses is due to a poor generation of KVV and CFD epitopes by proteasomes (9, 10).

To address the generation of CTL responses to the K^b-restricted Ova-derived epitopes, C57BL/6 mice were vaccinated with Ova alone or in combination with the Tat protein. We then evaluated the presence of epitope-specific CTL responses in fresh splenocytes that were tested against EL4 target cells pulsed with the relevant peptides (Fig. 20). Splenocytes isolated from mice immunized with Ova alone recognised target cells pulsed with the SII epitope but did not recognise cells pulsed with the KVV or CFD epitopes, confirming that CTL responses are mainly directed against the immunodominant SII peptide epitope. In contrast, splenocytes isolated from mice vaccinated with the combination Ova/Tat recognized less efficiently the immunodominant SII epitope, whereas clearly recognised target cells presenting the subdominant KVV and the cryptic CFD epitopes, respectively. Control mice did not recognise any peptide-pulsed EL4 cells.

In vivo modulation of epitope-specific T cell responses against Env and Gag by the HIV-1 Tat protein.

To address the effect of Tat on epitope specific T cell responses directed to Gag and Env antigens, BALB/c mice were vaccinated with the HIV-1 Env or HIV-1 Gag proteins alone, or in combination with the Tat protein. The presence of peptide-specific T cell responses was evaluated by IFN-γ Elispot assays using fresh

splenocytes stimulated with pools of peptides spanning the entire sequence of Env and Gag proteins.

Fresh splenocytes isolated from mice immunized with Env, either alone or in combination with Tat, did not respond to stimulation with any of the Env-derived peptide pools (data not shown). Subsequently, splenocytes from Env-vaccinated mice were stimulated with the immunodominant K^d-restricted RGP CTL epitope (amino acid 311-320), or with pools of peptides covering the entire Env sequence. After 5 days, all cultures were tested for specificity by IFN-γ Elispot following stimulation with pools of peptides using a peptide-based matrix approach. Matrices consisted of pools of peptides in which each peptide was present in two separate pools (see Fig 21).

As shown in Figure 16, after immunization with Env alone, IFNγ responses were detected against Env pools 1, 5, 16, 17, 21, 22, 25, and 26. Similarly, after immunization with Tat + Env and with TatCys22 + Env, an IFNγ responses was detected against the same Env pools 1, 5, 16, 17, 21, 22, 25, and 26. However, in the presence of Tat wild-type and TatCys22, additional responses to Env pools 4, 7, 14, 15, 18, 19, 23 and 27 were detected.

These results indicate that Tat and TatCys22 generally broaden the immune response to Env.

Fresh splenocytes isolated from mice immunized, with Gag alone or with Gag and Tat, were stimulated with pools of peptides using a peptide-based matrix approach and assayed by IFN-γ Elispot. Matrices consisted of pools of peptides in which each peptide was present in two separate pools (see Fig 22), thus offering internal positive controls.

Responses were regarded as positive if they had at least three times the mean number of SFU in the control wells, and had to be $\geq$ 50 SFU/10^6 cells.

As shown in Fig. 17, mice immunized with Gag alone responded to pools 5, 6, 9, 10, 15, 16, 17 and 18, whereas mice immunized with both Gag and Tat responded to pools 3, 5, 6, 9, 10, 13, 15, 16, 18 and 19. Control mice did not respond to any of the pools (not shown). In previous studies it has been demonstrated that major K^d-restricted CTL responses to Gag are directed to AMQ peptide (amino acid 197-205) contained in pools 5, 6 and 16, and to TTS peptide (amino acid 239-247) contained in pools 4, 5, and 17.

Interestingly, splenocytes from mice vaccinated with Tat and Gag, as compared to mice vaccinated with Gag alone, did not respond to pool 17 (containing the TTS peptide), whereas they recognized pools 13 and 19. We then assayed 36 individual peptides identified as potential targets by the matrix approach. As shown in Fig.18 , splenocytes from mice immunized with Gag alone responded to 6 different peptides (Gag42, Gag49, Gag50, Gag65, Gag75, Gag76), four of which (Gag49 and Gag50; Gag75 and Gag76) may contain 2 different overlapping peptides suggesting that T cell responses induced by Gag vaccination are directed to 4 different T cell epitopes. In contrast, mice immunized with Gag and Tat responded to 7 different peptides (Gag20, Gag39, Gag42, Gag49, Gag69, Gag76, Gag80) suggesting that T cell responses induced by Gag+Tat vaccination are directed to 7 different T cell epitopes, three more than vaccination with Gag alone.

In vivo modulation of epitope-specific T cell responses against Env and Gag by a mutated Tat protein (Tatcys22).

In the next set of experiments we evaluated the effect a Tat mutant carrying a glycine at position 22 instead of cysteine (referred to as Tatcys22). Tatcys22, in contrast to wild-type Tat, has no effect on the transactivation of the HIV-1 LTR, and does not induce reactivation of latent infection.

To address the effect of Tatcys22 on epitope specific T cell responses directed to Gag, BALB/c mice were also vaccinated with HIV-1 Gag protein alone, or in combination with the Tatcys22 protein, and assayed as previously described.

As shown in Fig. 19, splenocytes isolated from mice immunized with Gag and Tatcys22 recognised more peptide pools than splenocytes from mice immunized with

Gag alone. In particular, Tatcys22 induces Gag-specific responses directed to pools 3, 8, 13 and 19, which were not recognized after immunization with Gag alone. As previously, we then assayed 36 individual peptides (Fig. 20) identified by the matrix approach and we found that mice immunized with Gag in combination with Tatcys22 recognised 16 different peptides (Gag20, Gag21, Gag39, Gag42, Gag49, Gag50, Gag53, Gag60, Gag61, Gag64, Gag65, Gag69, Gag74, Gag75, Gag76, Gag80), 10 of which (Gag20 and Gag21; Gag49 and Gag50; Gag60 and Gag61; Gag64 and 65; Gag75 and Gag76) may contain 5 different overlapping peptides, suggesting that T cell responses induced by vaccination with Gag+Tatcys22 are directed to 11 different T cell epitopes, 7 more than mice immunized with Gag alone, and 4 more than mice immunized with Gag and wild-type Tat.

Conclusions

We have shown that native HIV-1 Tat protein and the mutant Tatcys22 protein modulate in vivo epitope specific T cell responses to the HIV-1 Gag and Env antigens. In particular, we have demonstrated that mice vaccinated with Gag, in combination with wild-type Tat or with the mutant Tatcys22, responded to 7 or 11 T cell Gag-derived epitopes respectively, in contrast to mice vaccinated with Gag alone, which responded to 4 T cell Gag-derived epitopes. Similarly, mice vaccinated with Env, in combination with wild-type Tat or with the mutant Tatcys22 responded to 12 Env-derived pools of peptides epitopes in contrast to mice vaccinated with Env alone which responded to 8 T cell Env-derived peptide pools.

These observations, together with our previous findings (2, 3, 11, 12), suggest that Tat is not only an antigen but also a novel adjuvant capable of increasing T cell responses against heterologous antigens. Therefore, the Tat protein, as well as mutant Tatcys22, may represent an important tool in HIV-1 vaccine strategies aimed at broadening the spectrum of the epitopes recognized by T cells.

References for Example 3

1. Wu, Y., and J. W. Marsh. 2003. Gene transcription in HIV infection. *Microbes Infect. 5:1023.*

2. Fanales-Belasio, E., S. Moretti, F. Nappi, G. Barillari, F. Micheletti, A. Cafaro, and B. Ensoli. 2002. Native HIV-1 Tat protein is selectively taken up by monocyte-derived dendritic cells and induces their maturation, Th-1 cytokine production and antigen presenting function. *J. Immunol. 168:197.*

3. Gavioli, R., E. Gallerani, C. Fortini, M. Fabris, A. Bottoni, A. Canella, A. Bonaccorsi, M. Marastoni, F. Micheletti, A. Cafaro, P. Rimessi, A. Caputo, and B. Ensoli. 2004. HIV-1 Tat protein modulates cytotoxic T cell epitopes by modifying proteasome composition and enzymatic activity. *J. Immunol. 173:3838.*

4. Ensoli, B., L. Buonaguro, G. Barillari, V. Fiorelli, R. Gendelman, R. A. Morgan, P. Wingfield, and R. C. Gallo. 1993. Release, uptake, and effect of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol. 67:277.*

5. Micheletti, F., A. Canella, S. Vertuani, M. Marastoni, L. Tosi, S. Volinia, S. Traniello, and R. Gavioli. 2000. Supra-agonist peptides enhance the reactivation of memory cytotoxic T lymphocyte responses. *J. Immunol. 165:4264.*

6. Gavioli, R., M. G. Kurilla, P. O. de Campos-Lima, L. E. Wallace, R. Dolcetti, R. J. Murray, A. B. Rickinson, and M. G. Masucci. 1993. Multiple HLA A11-restricted cytotoxic T-lymphocyte epitopes of different immunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. *J. Virol. 67:1572.*

7. Chen, W., S. Khilko, J. Fecondo, D. H. Margulies, and J. McCluskey. 1994. Determinant selection of major histocompatibility complex class I-restricted antigenic peptides is explained by class I-peptide affinity and is strongly influenced by nondominant anchor residues. *J. Exp. Med. 180:1471.*

8. Lipford, G. B., M. Hoffman, H. Wagner, and K. Heeg. 1993. Primary in vivo responses to ovalbumin. *J. Immunol. 150:1212.*

9. Niedermann, G., S. Butz, H. G. Ihlenfeldt, R. Grimm, M. Lucchiari, H. Hoschützky, G. Jung, B. Maier, and K. Eichmann. 1995. Contribution of proteasome-mediated proteolysis to the hierarchy of epitopes presented by major histocompatibility complex class I molecules. *Immunity 2:289.*

10. Mo, A. X. Y., S. F. L. van Lelyveld, A. Craiu, and K. L. Rock. 2000. Sequences that flank subdominant and cryptic epitopes influence the proteolytic generation of MHC class I-presented peptides. *J. Immunol. 164:4003.*

11. Cafaro, A., A. Caputo, C. Fracasso, M. T. Maggiorella, D. Goletti, S. Baroncelli, M. Pace, L. Sernicola, M. L. Koanga-Mogtomo, M. Betti, A. Borsetti, R. Belli, L. Åkerblom, F. Corrias, S. Butto, J. Heeney, P. Verani, F. Titti, and B. Ensoli. 1999. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nat. Med. 5:643.*

12. Cafaro, A., F. Titti, C. Fracasso, M. T. Maggiorella, S. Baroncelli, A. Caputo, D. Goletti, A. Borsetti, M. Pace, E. Fanales-Belasio, B. Ridolfi, D. R. Negri, L. Sernicola, R. Belli, F. Corrias, I. Macchia, P. Leone, Z. Michelini, P. ten Haaft, S. Butto, P. Verani, and B. Ensoli. 2001. Vaccination with DNA containing tat coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P). *Vaccine 19:2862.*

Claims:

1 Use of Tat, a biologically active equivalent, or a precursor therefor, in the preparation of a vaccine suitable to elicit an immune response against an antigenic substance having a plurality of epitopes, the epitopes including both immunodominant and sub-dominant epitopes, the vaccine comprising at least a part of the antigenic substance encoding or comprising a sub-dominant epitope thereof.

2 Use of Tat, a biologically active equivalent, thereof or a precursor therefor, in the preparation of a vaccine suitable to elicit an immune response against a plurality of strains of an infectious organism, the vaccine comprising antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope.

3 Use according to claim 1 or 2, wherein Tat is that shown in SEQ ID NO. 284.

4 Use according to claim 1 or 2, wherein Tat is a mutant and/or is a fragment of that shown in SEQ ID No. 284.

5 Use according to claim 4, wherein the mutant Tat has 90% homology to SEQ ID NO. 284.

6 Use according to claim 4 or 5, wherein Tat is mutated at position 22 of SEQ ID NO. 284.

7 Use according to claim 6, wherein a Cysteine residue is present at position 22 and is substituted by glycine

8 Use according to claim 1 or 2, wherein a fragment of Tat is used, comprising or encoding amino acid numbers 47-86 of SEQ ID NO 284.

9 Use according to claim 9, wherein the fragment is a polypeptide consisting of amino acid numbers 47-86 of SEQ ID NO 284.

10 Use according to any preceding claim, wherein the vaccine comprises an expression sequence for Tat together with a vector therefor, said expression sequence being suitable to express Tat in a target cell.

11 Use according to claim 10, wherein Tat is under the control of an inducible promoter.

12 Use according to any of claims 1-9, wherein Tat is provided as a peptide or protein.

13 Use according to any preceding claim, wherein the antigen is derived from or comprises HIV.

14. Use according to any of claims 1-12, wherein the antigen is derived from or comprises influenza or SARS.

15. Use according to claim 1, wherein the antigenic substance is associated with a tumour or immunomediated disease.

16 Use according to any of claims 1-12, wherein the antigen is derived from plants, parasites, fungi or bacteria.

17 Use according to claim 16, wherein the antigen is derived from or comprises Mycobacteria.

18 Use according to claim 13, wherein the peptide is derived from or comprises Gag, or a fragment thereof.

19 Use according to claim 13, wherein the peptide is derived from or comprises Env, or a fragment thereof.

20 Use according to any preceding claim, wherein the vaccine is administered orally, intravenously, intramuscularly, intraperitonealy, transdermally, or subcutaneously.

21 Use according to any preceding claim, wherein the vaccine is used in a prime-boost regimen.

22 Use according to any preceding claim, wherein the Tat, its equivalent, or precursor, is capable of down-regulating levels of LMP2 in the intended recipient of the vaccine.

23 Use of a vaccine for modulating proteosome subunit composition, by administering Tat to down-regulate expression of the LMP2 subunit.

24 A vaccine for eliciting an immune response against an antigenic substance having a plurality of epitopes, the vaccine comprising Tat, a biologically active equivalent, or a precursor therefor, the epitopes including both immunodominant and sub-dominant epitopes, and at least a part of the antigenic substance encoding or comprising a sub-dominant epitope thereof.

25 A vaccine for eliciting an immune response against a plurality of strains of an infectious organism, the vaccine comprising Tat, a biologically active equivalent, thereof or a precursor therefore, and antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope.

26 A vaccine according to claim 24 or 25, wherein the Tat is that shown in SEQ ID NO. 284.

27 A vaccine for use in any of claims 1-23.

28 A vaccine according to any of claims 24-26, wherein Tat and the antigen are provided as proteins or peptides.

29 Use of a vaccine according to any of claims 24-28 to stimulate cross-strain immunity.

30 A vaccine comprising Tat and an antigen, as defined in any of claims 1-23, and a vehicle therefor.

31 A method for providing an immune response against a plurality of strains of an infectious organism, comprising administering a vaccine comprising:
antigenic material from at least one strain of the organism, said material encoding or comprising a subdominant epitope; and
Tat, a biologically active equivalent, thereof or a precursor therefor.

32 A method according to claim 31, wherein the Tat is a mutant or fragment of SEQ ID NO 284.

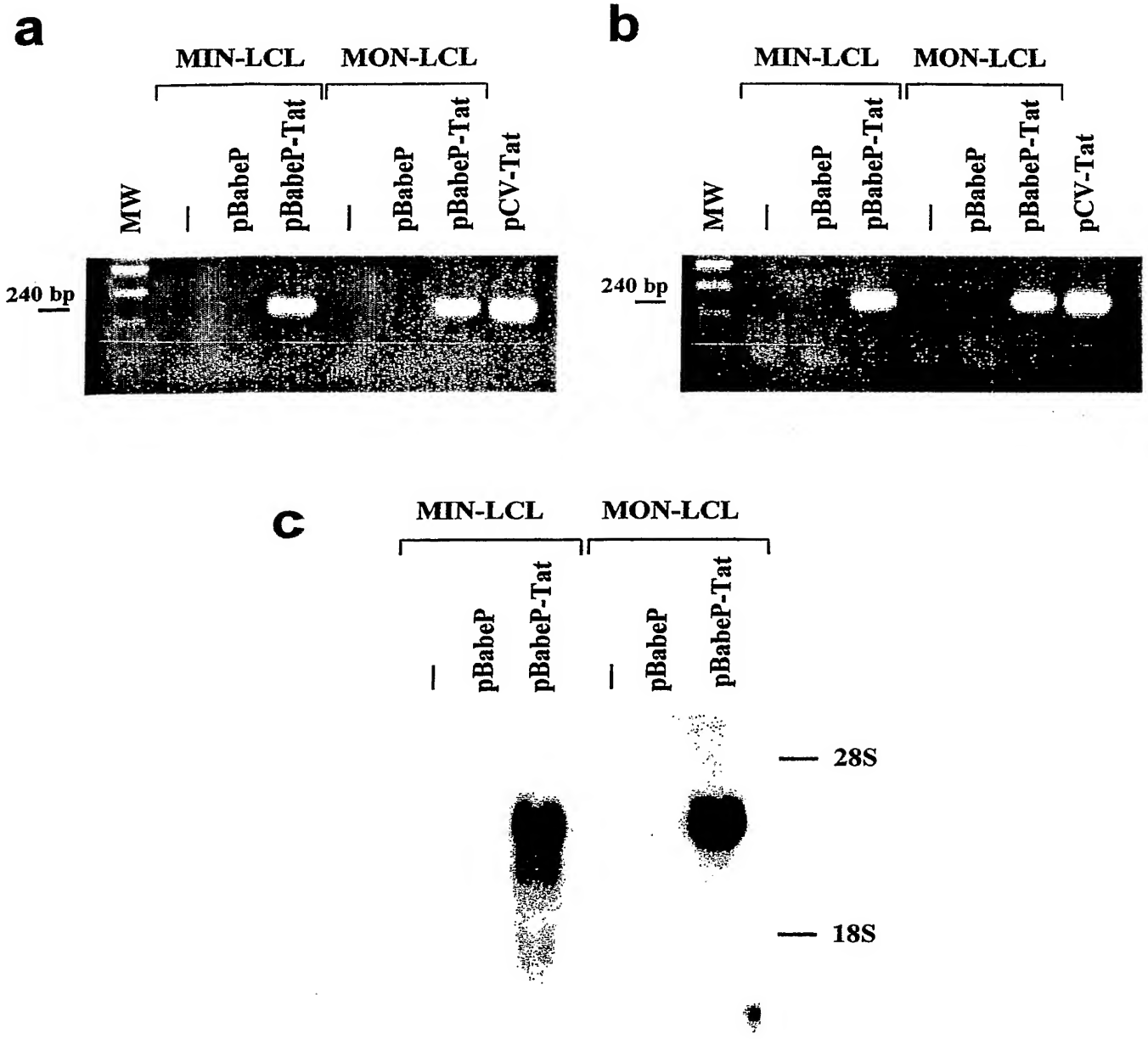
a
MIN-LCL
MON-LCL
MW
–
pBabeP
pBabeP-Tat
–
pBabeP
pBabeP-Tat
pCV-Tat
240 bp
b
MIN-LCL
MON-LCL
MW
–
pBabeP
pBabeP-Tat
–
pBabeP
pBabeP-Tat
pCV-Tat
240 bp
c
MIN-LCL
MON-LCL
–
pBabeP
pBabeP-Tat
–
pBabeP
pBabeP-Tat
28S
18S

FIG. 1

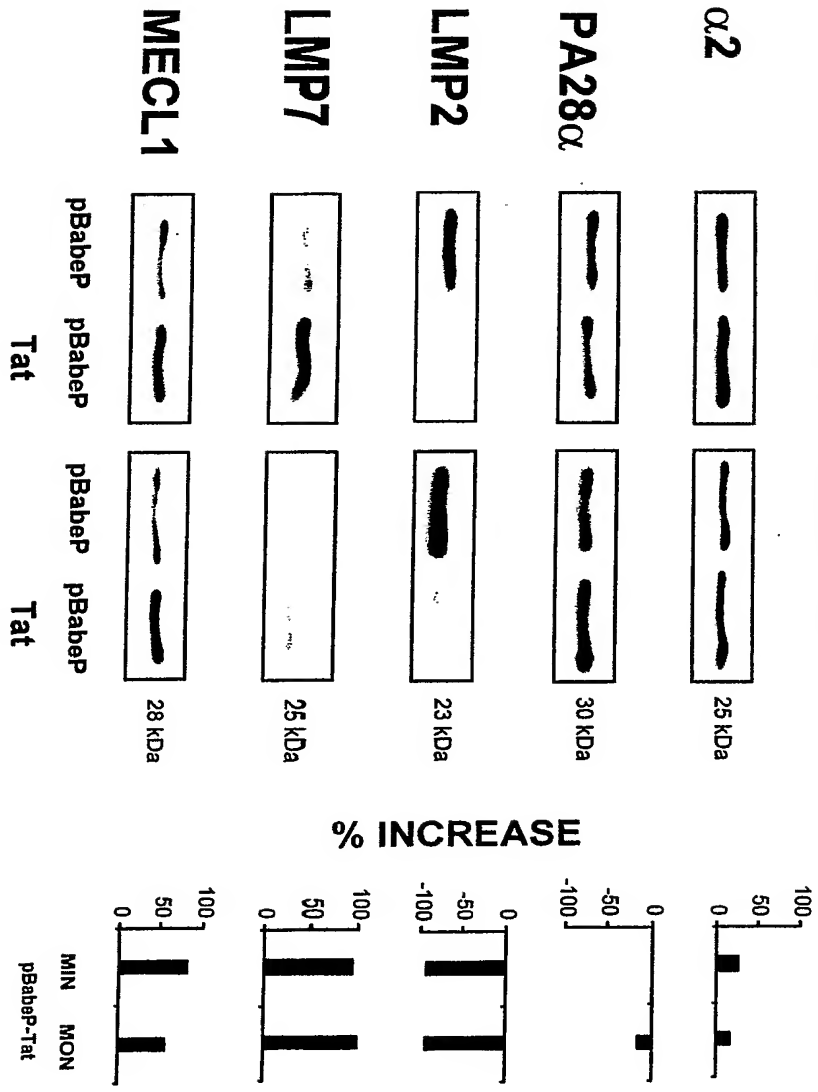
MIN-LCL
MON-LCL
α2
PA28α
LMP2
LMP7
MECL1
pBabeP
pBabeP-Tat
25 kDa
30 kDa
23 kDa
25 kDa
28 kDa
% INCREASE
MIN
MON

FIG. 2

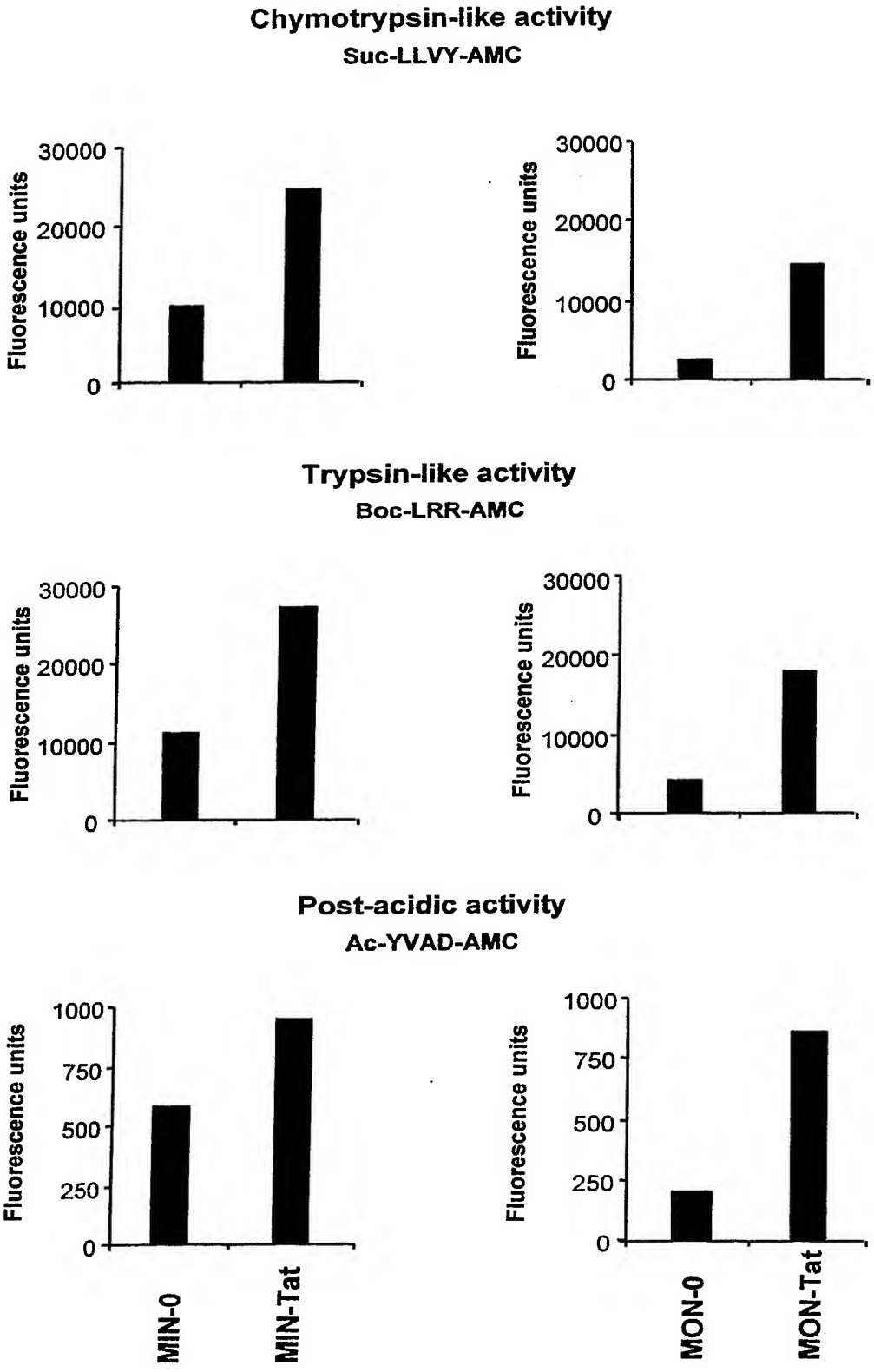
Chymotrypsin-like activity
Suc-LLVY-AMC
Fluorescence units
30000
20000
10000
0
Trypsin-like activity
Boc-LRR-AMC
Post-acidic activity
Ac-YVAD-AMC
1000
750
500
250
MIN-0
MIN-Tat
MON-0
MON-Tat

FIG. 3

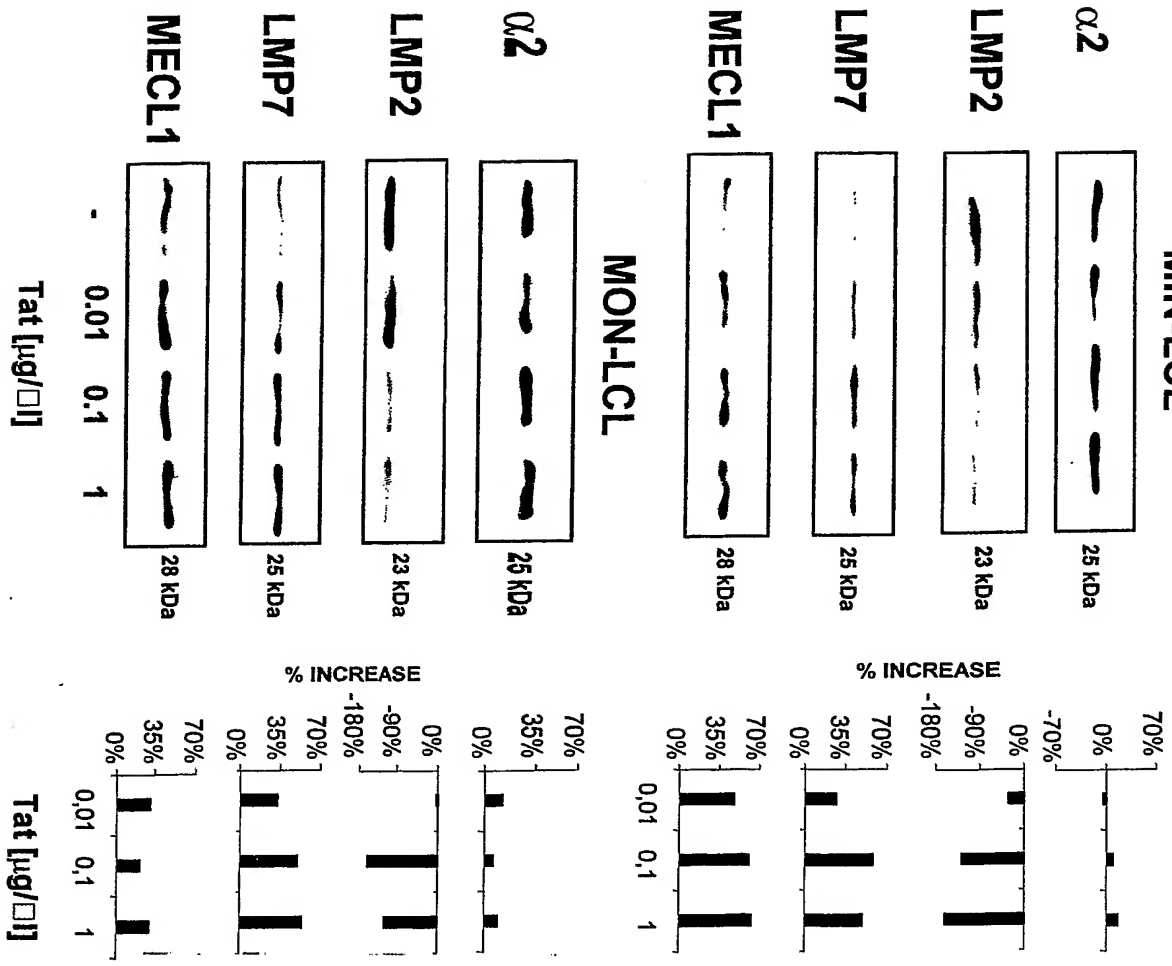
MIN-LCL
α2
LMP2
LMP7
MECL1
25 kDa
23 kDa
25 kDa
28 kDa
% INCREASE
70%
0%
-70%
-90%
-180%
35%
0,01
0,1
1
MON-LCL
α2
LMP2
LMP7
MECL1
25 kDa
23 kDa
25 kDa
28 kDa
% INCREASE
Tat [µg/□l]
-
0.01
0.1
1

FIG. 4

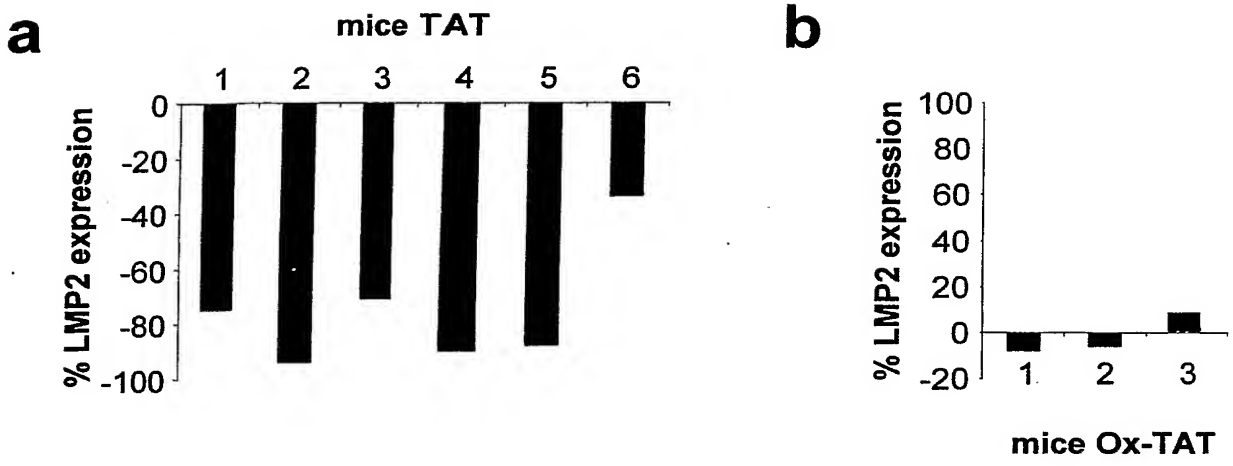
a
mice TAT
1
2
3
4
5
6
0
-20
-40
-60
-80
-100
% LMP2 expression
b
100
80
60
40
20
0
-20
1
2
3
% LMP2 expression
mice Ox-TAT

FIG. 5

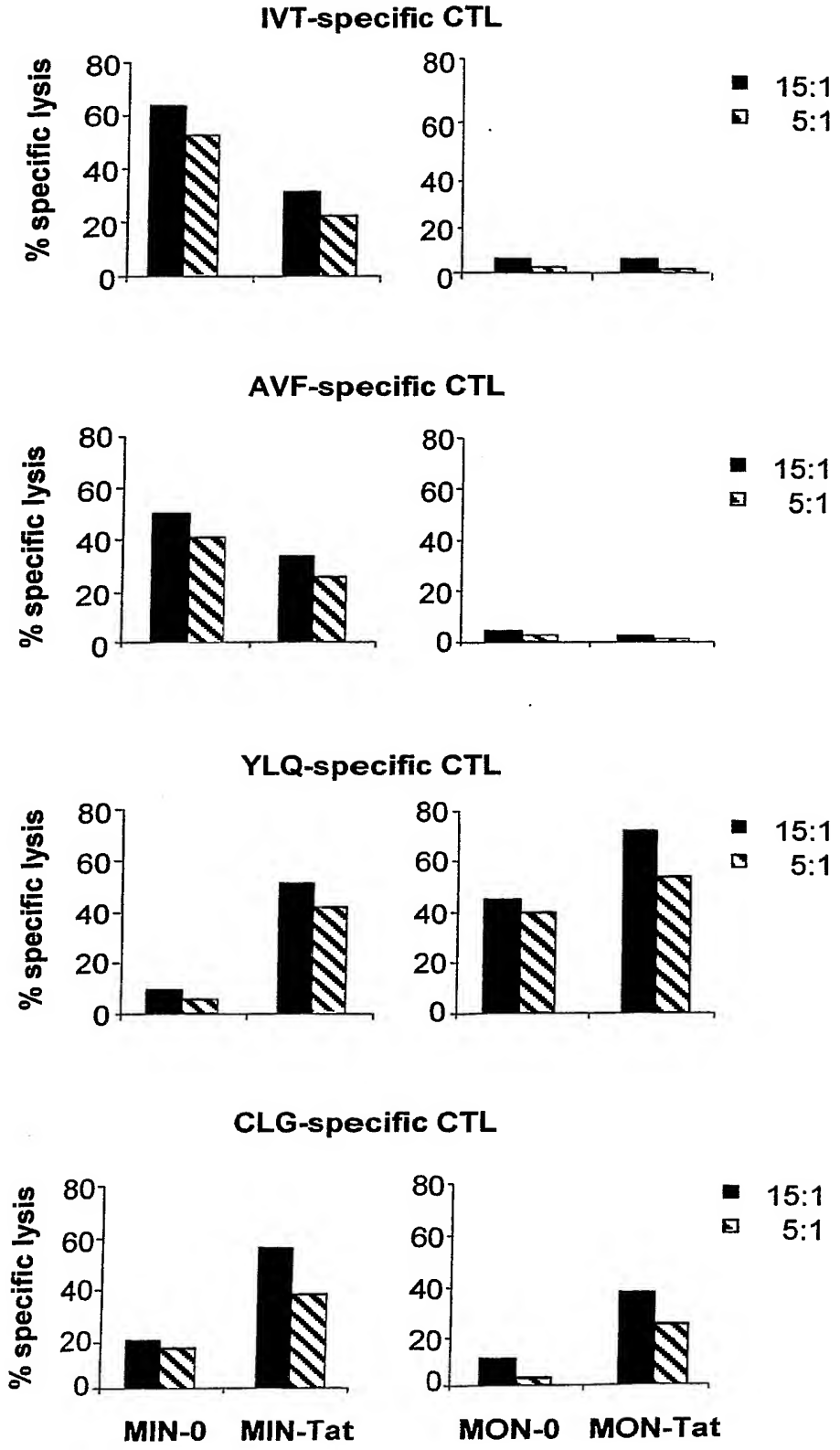
IVT-specific CTL
AVF-specific CTL
YLQ-specific CTL
CLG-specific CTL
% specific lysis
0
20
40
60
80
15:1
5:1
MIN-0
MIN-Tat
MON-0
MON-Tat

FIG. 6

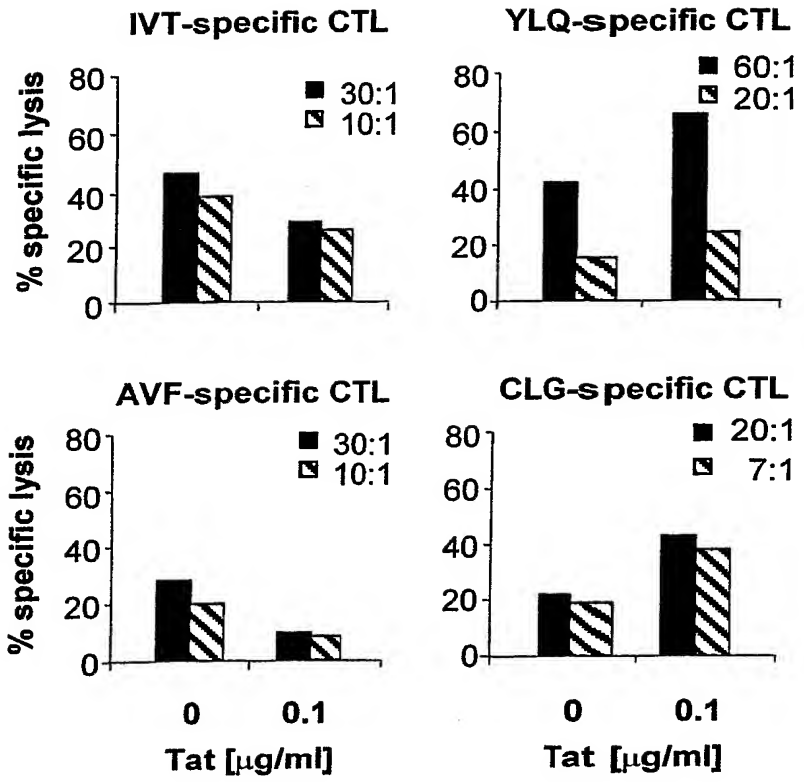
IVT-specific CTL
YLQ-specific CTL
AVF-specific CTL
CLG-specific CTL
% specific lysis
80
60
40
20
0
30:1
10:1
60:1
20:1
20:1
7:1
0
0.1
Tat [µg/ml]

FIG. 7

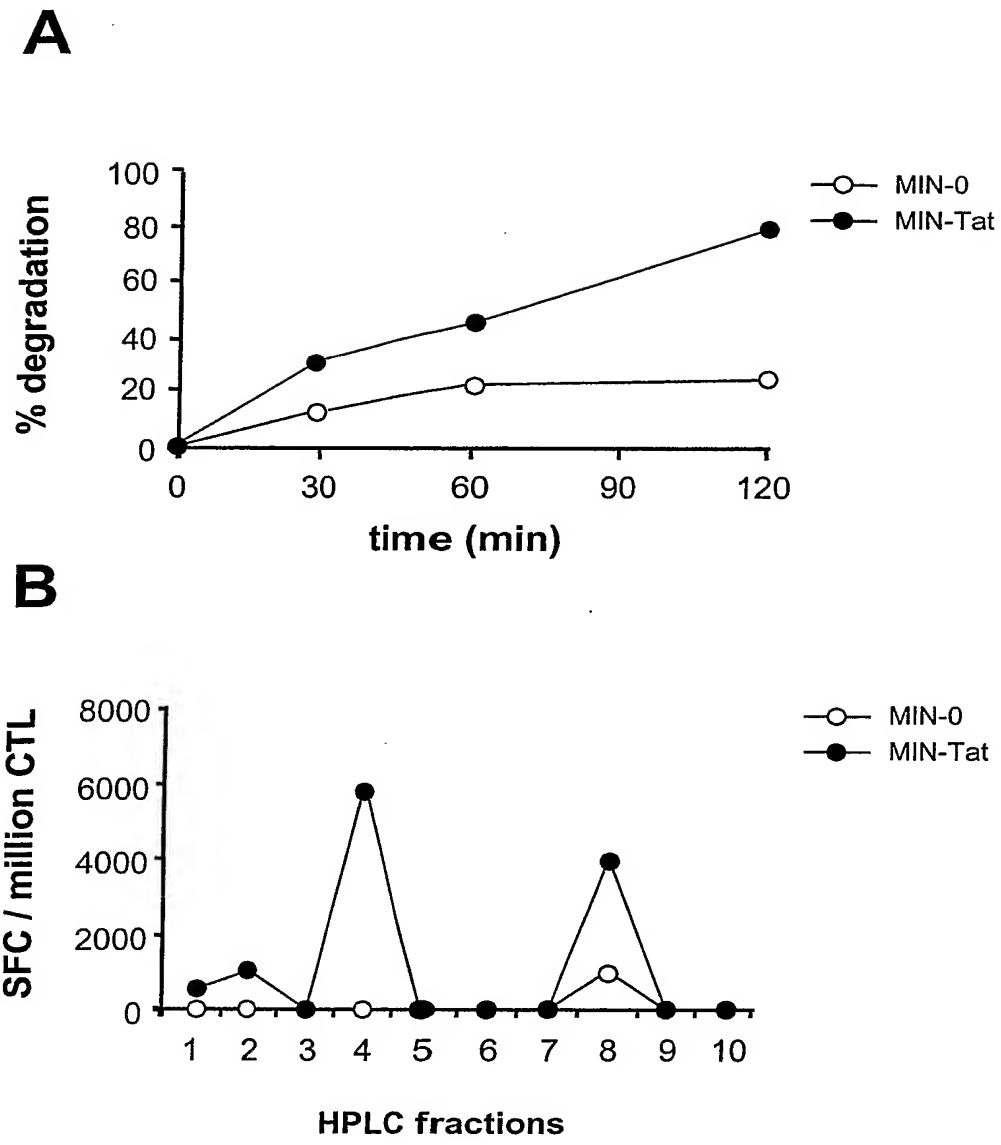
A
100
80
60
40
20
0
% degradation
0
30
60
90
120
time (min)
MIN-0
MIN-Tat
B
8000
6000
4000
2000
0
SFC / million CTL
1
2
3
4
5
6
7
8
9
10
HPLC fractions
MIN-0
MIN-Tat

FIG. 8

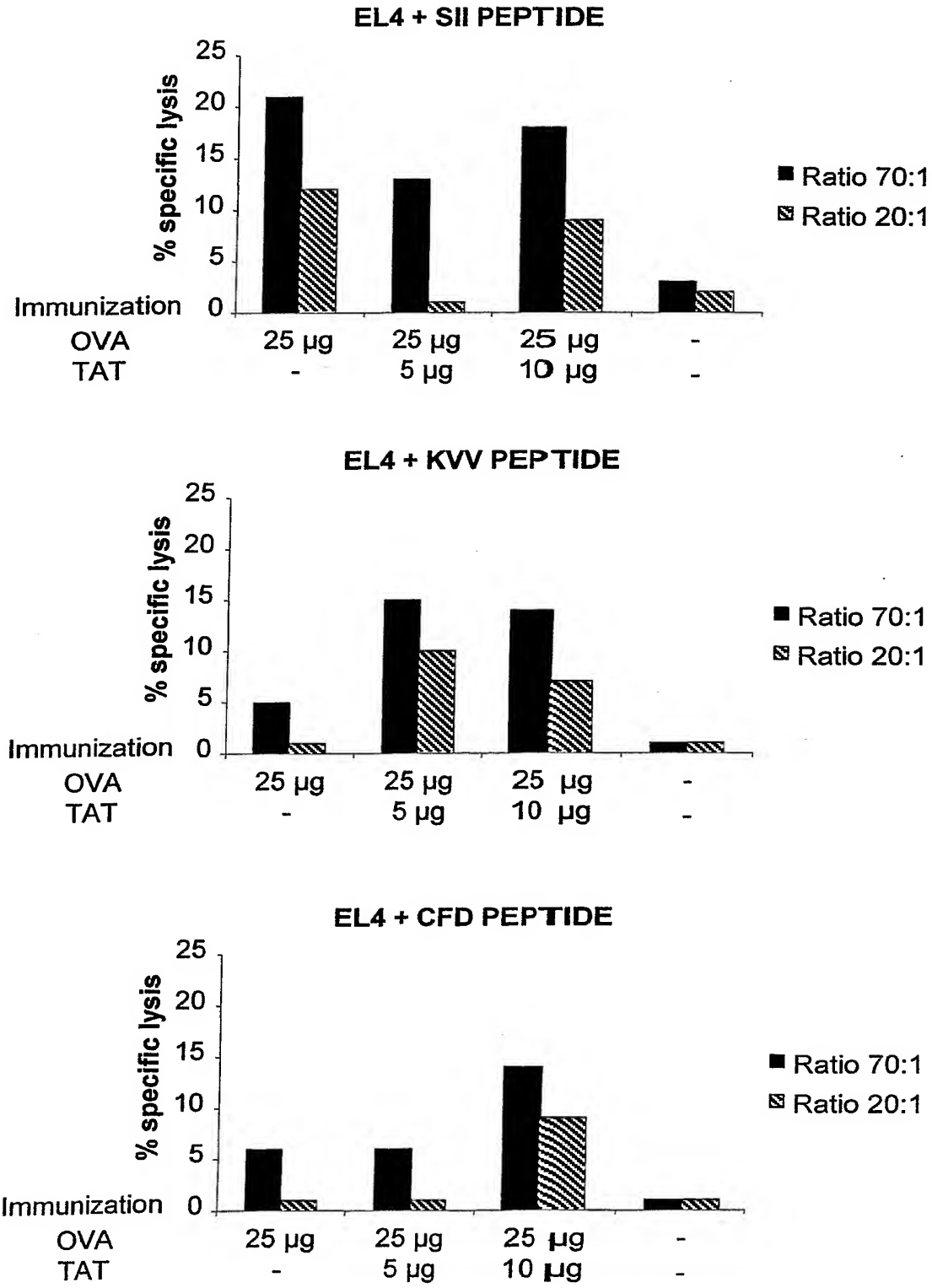
EL4 + SII PEPTIDE
% specific lysis
25
20
15
10
5
0
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1
EL4 + KVV PEPTIDE
% specific lysis
25
20
15
10
5
0
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1
EL4 + CFD PEPTIDE
% specific lysis
25
20
15
10
5
0
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1

FIG. 9

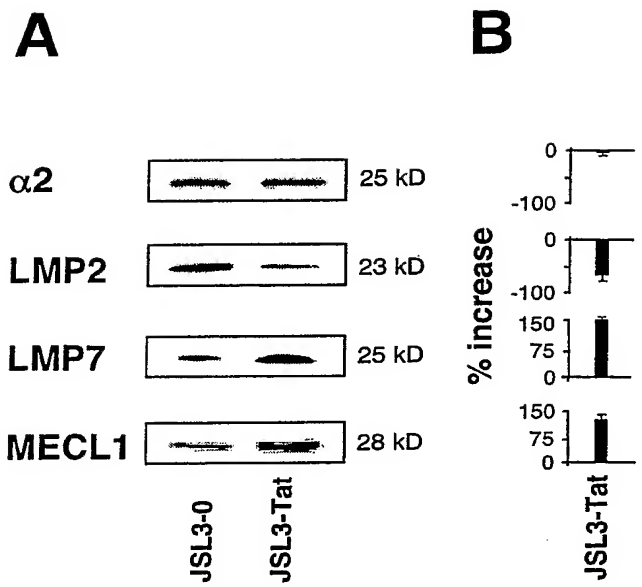
A
B
α2
LMP2
LMP7
MECL1
25 kD
23 kD
25 kD
28 kD
JSL3-0
JSL3-Tat
% increase
0
-100
0
-100
150
75
0
150
75
0
JSL3-Tat

FIG. 10

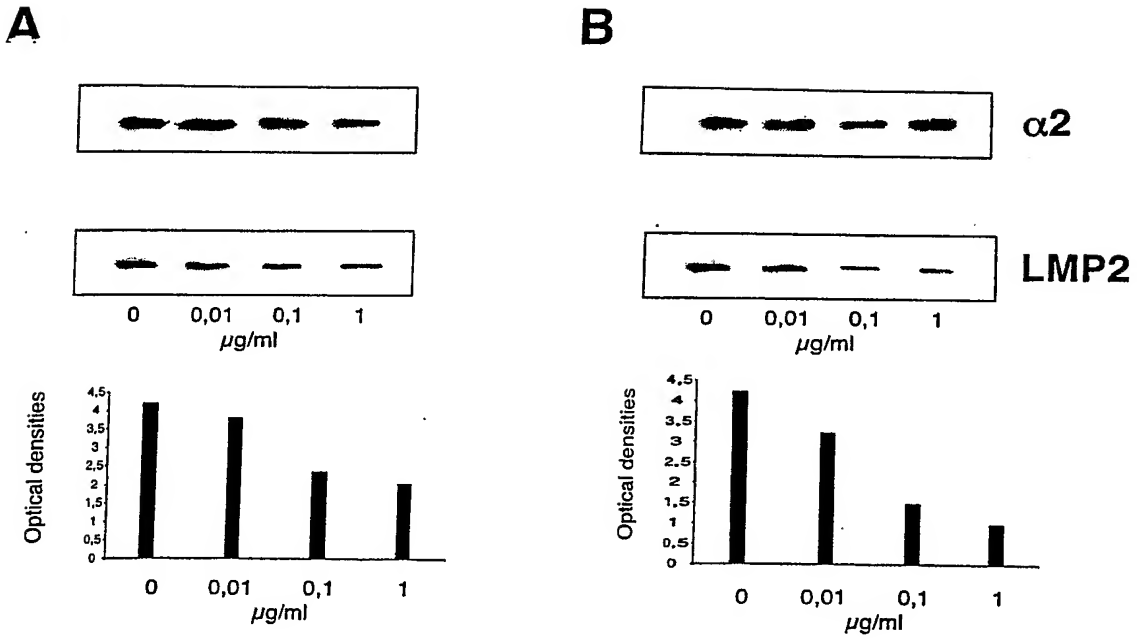
A
B
α2
LMP2
0 0,01 0,1 1
µg/ml
Optical densities
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0

FIG. 11

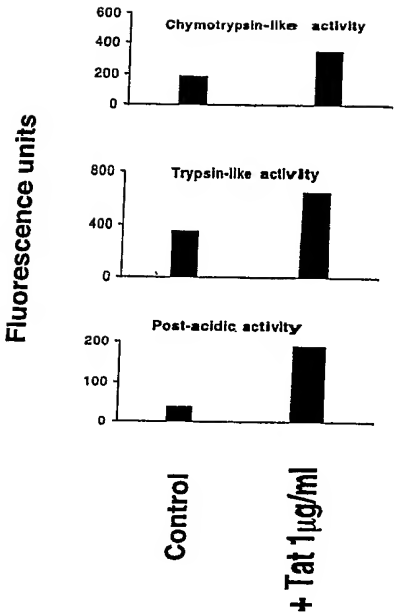
Chymotrypsin-like activity
600
400
200
0
Trypsin-like activity
800
400
0
Post-acidic activity
200
100
0
Fluorescence units
Control
+ Tat 1µg/ml

FIG. 12

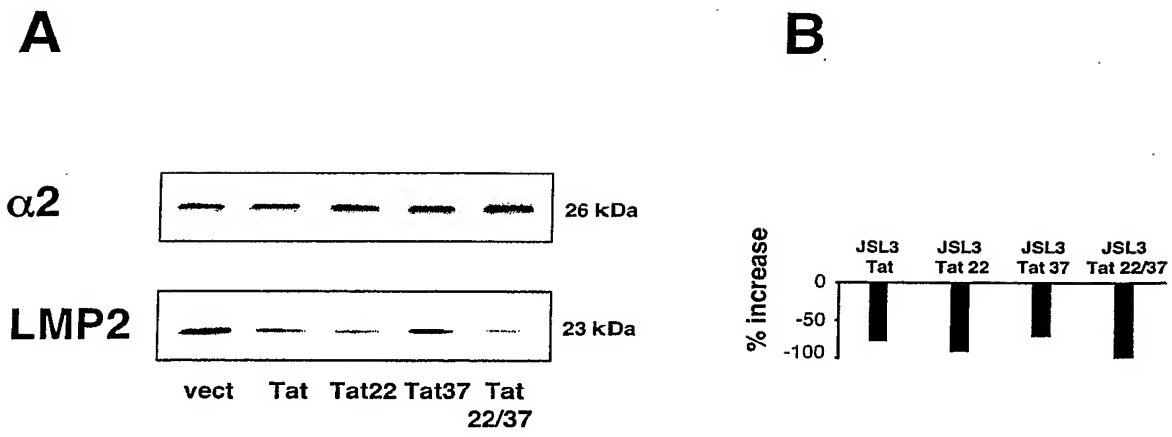
A
α2
26 kDa
LMP2
23 kDa
vect
Tat
Tat22
Tat37
Tat 22/37
B
% increase
0
-50
-100
JSL3 Tat
JSL3 Tat 22
JSL3 Tat 37
JSL3 Tat 22/37

FIG. 13

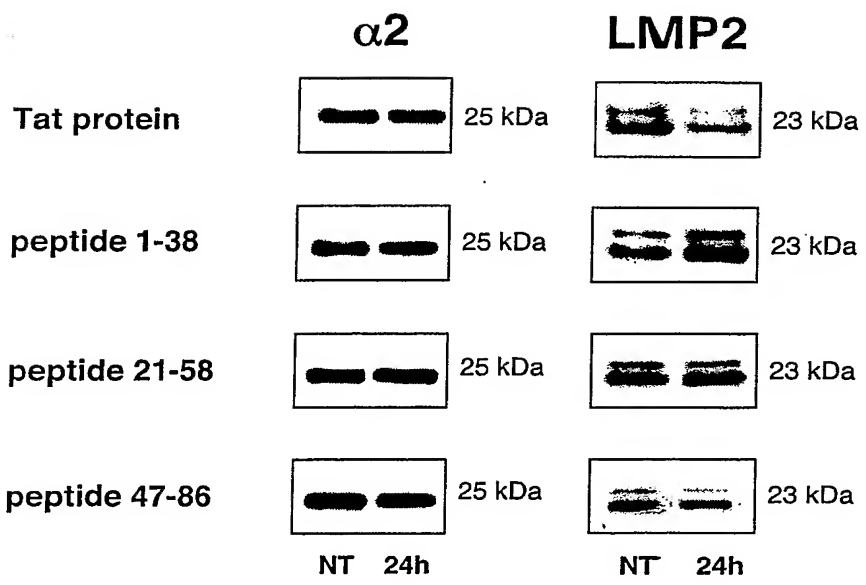
α2
LMP2
Tat protein
25 kDa
23 kDa
peptide 1-38
25 kDa
23 kDa
peptide 21-58
25 kDa
23 kDa
peptide 47-86
25 kDa
23 kDa
NT 24h
NT 24h

FIG. 14

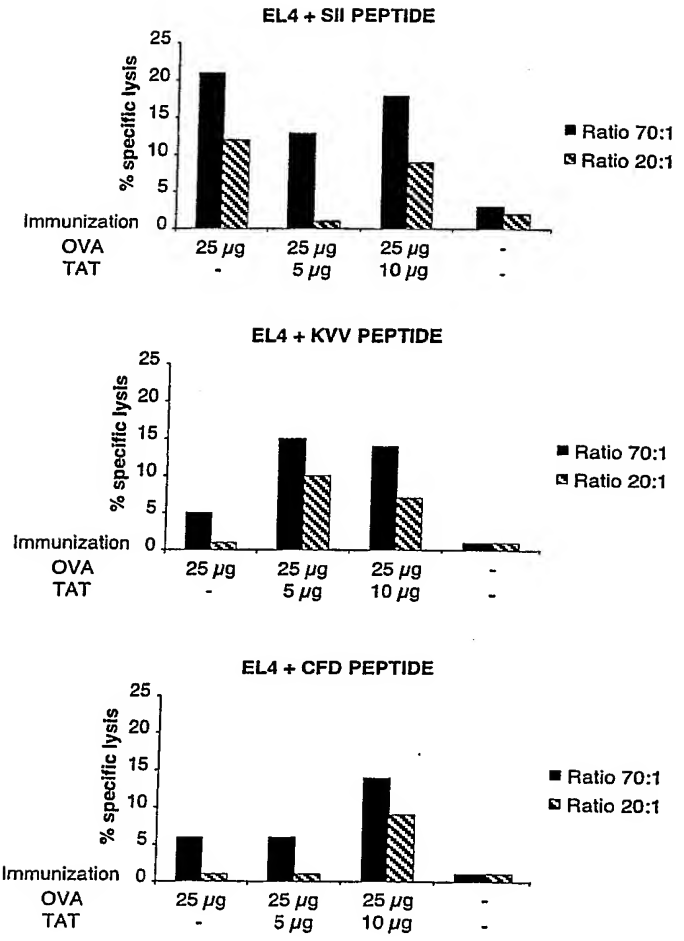
EL4 + SII PEPTIDE
% specific lysis
25
20
15
10
5
0
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1
EL4 + KVV PEPTIDE
% specific lysis
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1
EL4 + CFD PEPTIDE
% specific lysis
Immunization
OVA
TAT
25 µg
-
25 µg
5 µg
25 µg
10 µg
-
-
Ratio 70:1
Ratio 20:1

FIG. 15

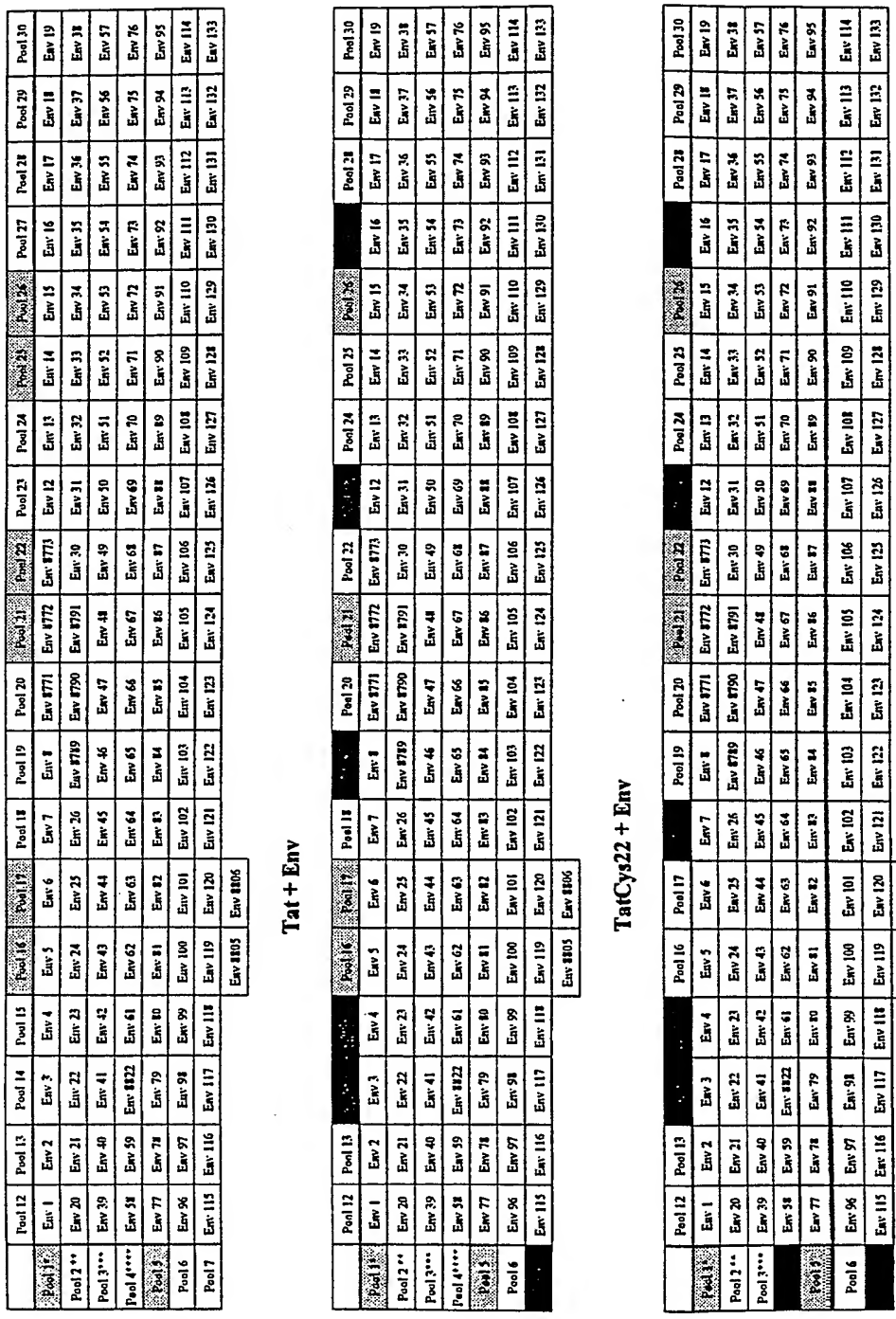

FIG. 16

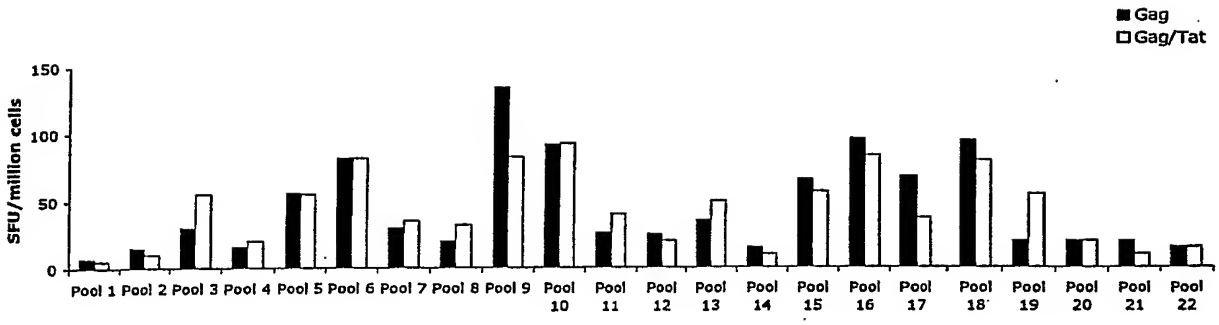
Gag
Gag/Tat
SFU/million cells
150
100
50
0
Pool 1
Pool 2
Pool 3
Pool 4
Pool 5
Pool 6
Pool 7
Pool 8
Pool 9
Pool 10
Pool 11
Pool 12
Pool 13
Pool 14
Pool 15
Pool 16
Pool 17
Pool 18
Pool 19
Pool 20
Pool 21
Pool 22

FIG. 17

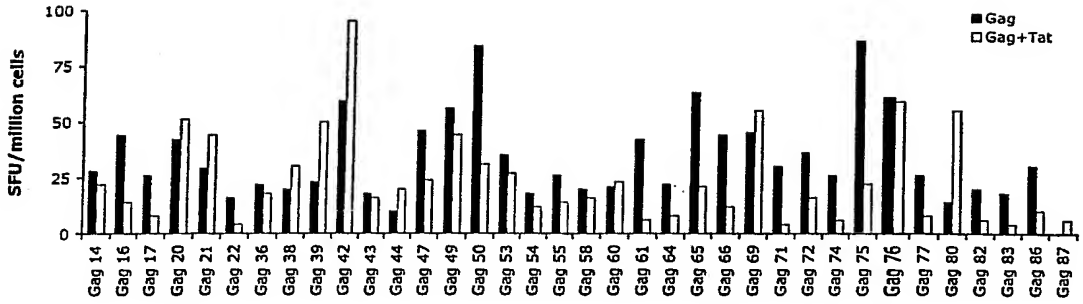
SFU/million cells
100
75
50
25
0
Gag
Gag+Tat
Gag 14
Gag 16
Gag 17
Gag 20
Gag 21
Gag 22
Gag 36
Gag 38
Gag 39
Gag 42
Gag 43
Gag 44
Gag 47
Gag 49
Gag 50
Gag 53
Gag 54
Gag 55
Gag 58
Gag 60
Gag 61
Gag 64
Gag 65
Gag 66
Gag 69
Gag 71
Gag 72
Gag 74
Gag 75
Gag 76
Gag 77
Gag 80
Gag 82
Gag 83
Gag 86
Gag 87

FIG. 18

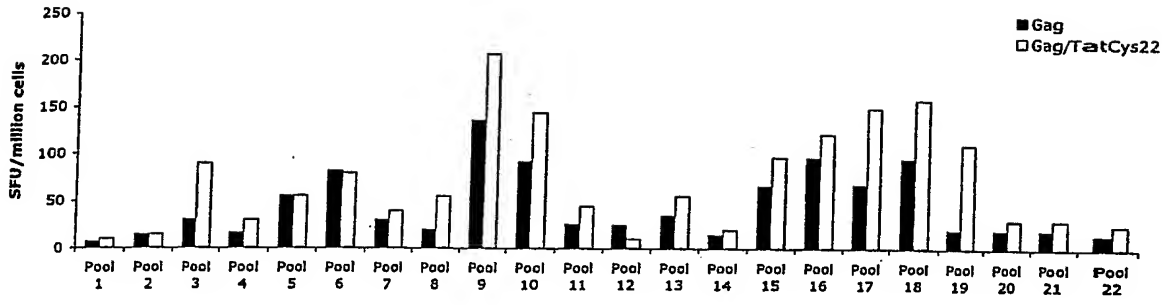
SFU/million cells
250
200
150
100
50
0
Gag
Gag/TatCys22
Pool 1
Pool 2
Pool 3
Pool 4
Pool 5
Pool 6
Pool 7
Pool 8
Pool 9
Pool 10
Pool 11
Pool 12
Pool 13
Pool 14
Pool 15
Pool 16
Pool 17
Pool 18
Pool 19
Pool 20
Pool 21
Pool 22

FIG. 19

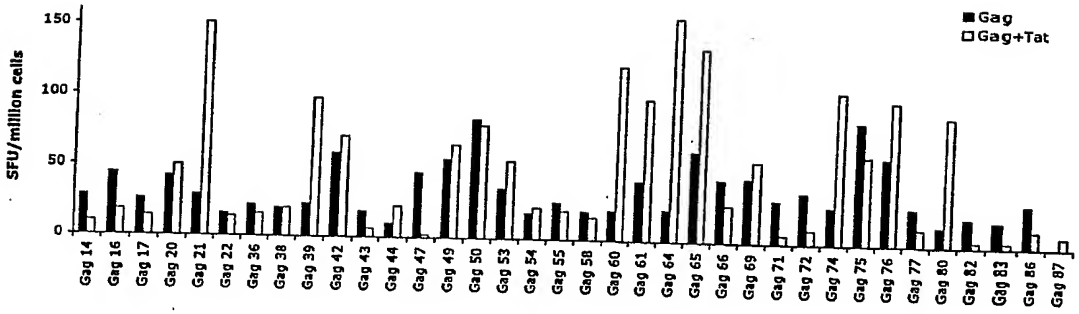
SFU/million cells
150
100
50
0
Gag
Gag+Tat
Gag 14
Gag 16
Gag 17
Gag 20
Gag 21
Gag 22
Gag 36
Gag 38
Gag 39
Gag 42
Gag 43
Gag 44
Gag 47
Gag 49
Gag 50
Gag 53
Gag 54
Gag 55
Gag 58
Gag 60
Gag 61
Gag 64
Gag 65
Gag 66
Gag 69
Gag 71
Gag 72
Gag 74
Gag 75
Gag 76
Gag 77
Gag 80
Gag 82
Gag 83
Gag 86
Gag 87

FIG. 20

	Pool 12	Pool 13	Pool 14	Pool 15	Pool 16	Pool 17	Pool 18	Pool 19	Pool 20	Pool 21	Pool 22	Pool 23	Pool 24	Pool 25	Pool 26	Pool 27	Pool 28	Pool 29	Pool 30
Pool 1	Env 1	Env 2	Env 3	Env 4	Env 5	Env 6	Env 7	Env 8	Env 8771	Env 8772	Env 8773	Env 12	Env 13	Env 14	Env 15	Env 16	Env 17	Env 18	Env 19
Pool 2	Env 20	Env 21	Env 22	Env 23	Env 24	Env 25	Env 26	Env 8789	Env 8790	Env 8791	Env 30	Env 31	Env 32	Env 33	Env 34	Env 35	Env 36	Env 37	Env 38
Pool 3	Env 39	Env 40	Env 41	Env 42	Env 43	Env 44	Env 45	Env 46	Env 47	Env 48	Env 48	Env 50	Env 51	Env 52	Env 53	Env 54	Env 55	Env 56	Env 57
Pool 4	Env 58	Env 59	Env 8822	Env 61	Env 62	Env 63	Env 64	Env 65	Env 66	Env 67	Env 68	Env 69	Env 70	Env 71	Env 72	Env 73	Env 74	Env 75	Env 76
Pool 5	Env 77	Env 78	Env 79	Env 80	Env 81	Env 82	Env 83	Env 84	Env 85	Env 86	Env 87	Env 88	Env 89	Env 90	Env 91	Env 92	Env 93	Env 94	Env 95
Pool 6	Env 96	Env 97	Env 98	Env 99	Env 100	Env 101	Env 102	Env 103	Env 104	Env 105	Env 106	Env 107	Env 108	Env 109	Env 110	Env 111	Env 112	Env 113	Env 114
Pool 7	Env 115	Env 116	Env 117	Env 118	Env 119	Env 120	Env 121	Env 122	Env 123	Env 124	Env 125	Env 126	Env 127	Env 128	Env 129	Env 130	Env 131	Env 132	Env 133
					Env 8805	Env 8806													

FIG. 21

	pool 1	pool 2	pool 3	pool 4	pool 5	pool 6	pool 7	pool 8	pool 9	pool 10	pool 11
pool 12	Gag 1	Gag 2	Gag 3	Gag 4	Gag 5	Gag 6	Gag 7	Gag 8	Gag 9	Gag 10	Gag 11
pool 13	Gag 12	Gag 13	Gag 14	Gag 15	Gag 16	Gag 17	Gag 18	Gag 19	Gag 20	Gag 21	Gag 22
pool 14	Gag 23	Gag 24	Gag 25	Gag 26	Gag 27	Gag 28	Gag 29	Gag 30	Gag 31	Gag 32	Gag 33
pool 15	Gag 34	Gag 35	Gag 36	Gag 37	Gag 38	Gag 39	Gag 40	Gag 41	Gag 42	Gag 43	Gag 44
pool 16	Gag 45	Gag 46	Gag 47	Gag 48	Gag 49	Gag 50	Gag 51	Gag 52	Gag 53	Gag 54	Gag 55
pool 17	Gag 56	Gag 57	Gag 58	Gag 59	Gag 60	Gag 61	Gag 62	Gag 63	Gag 64	Gag 65	Gag 66
pool 18	Gag 67	Gag 68	Gag 69	Gag 70	Gag 71	Gag 72	Gag 73	Gag 74	Gag 75	Gag 76	Gag 77
pool 19	Gag 78	Gag 79	Gag 80	Gag 81	Gag 82	Gag 83	Gag 84	Gag 85	Gag 86	Gag 87	Gag 88
pool 20	Gag 89	Gag 90	Gag 91	Gag 92	Gag 93	Gag 94	Gag 95	Gag 96	Gag 97	Gag 98	Gag 99
pool 21	Gag 100	Gag 101	Gag 102	Gag 103	Gag 104	Gag 105	Gag 106	Gag 107	Gag 108	Gag 109	Gag 110
pool 22	Gag 111	Gag 112	Gag 113	Gag 114	Gag 115	Gag 116	Gag 117	Gag 118	Gag 119	Gag 120	Gag 121	Gag 122	Gag 123
	Gag 122	Gag 123									

FIG. 22

SEQUENCE LISTING

<110> Istituto Superiore di Sanità

<120> Vaccines

<130> WPP88239

<150> GB 0323840.9
<151> 2003-10-10

<160> 288

<170> PatentIn version 3.3

<210> 1
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 1

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg
1 5 10 15

<210> 2
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 2

Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile
1 5 10 15

<210> 3
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 3

Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile Arg Leu Arg Pro
1 5 10 15

<210> 4
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 4

Leu Asp Arg Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys

1 5 10 15

<210> 5
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 5

Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu
1 5 10 15

<210> 6
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 6

Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val
1 5 10 15

<210> 7
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 7

Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg
1 5 10 15

<210> 8
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 8

Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg
1 5 10 15

<210> 9
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 9

His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn
1 5 10 15

<210> 10
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 10

Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu
1 5 10 15

<210> 11
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 11

Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu
1 5 10 15

<210> 12
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 12

Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln
1 5 10 15

<210> 13
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 13

Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln
1 5 10 15

<210> 14
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 14

Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser
1 5 10 15

<210> 15
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 15

Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly
1 5 10 15

<210> 16
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 16

Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu
1 5 10 15

<210> 17
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 17

Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr
1 5 10 15

<210> 18
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 18

Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala
1 5 10 15

<210> 19
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 19

Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys
1 5 10 15

<210> 20
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 20

Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg
1 5 10 15

<210> 21
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 21

Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Val Lys
1 5 10 15

<210> 22
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 22

Leu Tyr Cys Val His Gln Arg Ile Glu Val Lys Asp Thr Lys Glu
1 5 10 15

<210> 23
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 23

His Gln Arg Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Glu Lys
1 5 10 15

<210> 24
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Gag peptide

<400> 24

Glu Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu
1 5 10 15

<210> 25
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 25

Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser
1 5 10 15

<210> 26
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 26

Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala
1 5 10 15

<210> 27
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 27

Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala
1 5 10 15

<210> 28
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 28

Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly
1 5 10 15

<210> 29

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 29

Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly Asn Ser Ser Gln
1 5 10 15

<210> 30
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 30

Gln Ala Ala Ala Asp Thr Gly Asn Ser Ser Gln Val Ser Gln Asn
1 5 10 15

<210> 31
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 31

Asp Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val
1 5 10 15

<210> 32
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 32

Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln
1 5 10 15

<210> 33
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 33

Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val
1 5 10 15

<210> 34
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 34

Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile
1 5 10 15

<210> 35
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 35

Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr
1 5 10 15

<210> 36
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 36

Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp
1 5 10 15

<210> 37
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 37

Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val
1 5 10 15

<210> 38
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 38

Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala

1 5 10 15

<210> 39
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 39

Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu
1 5 10 15

<210> 40
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag peptide

<400> 40

Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met
1 5 10 15

<210> 41
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 41

Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu
1 5 10 15

<210> 42
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 42

Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala
1 5 10 15

<210> 43
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 43

Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp
1 5 10 15

<210> 44
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 44

Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met
1 5 10 15

<210> 45
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 45

Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val
1 5 10 15

<210> 46
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 46

Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln
1 5 10 15

<210> 47
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 47

Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln
1 5 10 15

<210> 48
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 48

Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu
1 5 10 15

<210> 49
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 49

Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu
1 5 10 15

<210> 50
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 50

Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu
1 5 10 15

<210> 51
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 51

Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu
1 5 10 15

<210> 52
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 52

Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His
1 5 10 15

<210> 53
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 53

Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile
1 5 10 15

<210> 54
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 54

Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln
1 5 10 15

<210> 55
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 55

Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro
1 5 10 15

<210> 56
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 56

Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp
1 5 10 15

<210> 57
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 57

Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr
1 5 10 15

<210> 58
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Gag Peptide

<400> 58

Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu
1 5 10 15

<210> 59
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 59

Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile
1 5 10 15

<210> 60
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 60

Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr
1 5 10 15

<210> 61
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 61

Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro
1 5 10 15

<210> 62
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 62

Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly
1 5 10 15

<210> 63

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 63

Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys
1 5 10 15

<210> 64
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 64

Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile
1 5 10 15

<210> 65
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 65

Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn
1 5 10 15

<210> 66
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 66

Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg
1 5 10 15

<210> 67
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 67

Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro
1 5 10 15

<210> 68
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 68

Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu
1 5 10 15

<210> 69
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 69

Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln
1 5 10 15

<210> 70
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 70

Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu
1 5 10 15

<210> 71
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 71

Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp
1 5 10 15

<210> 72
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 72

Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg

1 5 10 15

<210> 73
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 73

Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr
1 5 10 15

<210> 74
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 74

Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu
1 5 10 15

<210> 75
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 75

Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln
1 5 10 15

<210> 76
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 76

Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn
1 5 10 15

<210> 77
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 77

Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu
1 5 10 15

<210> 78
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 78

Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val
1 5 10 15

<210> 79
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 79

Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn
1 5 10 15

<210> 80
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 80

Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys
1 5 10 15

<210> 81
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 81

Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys
1 5 10 15

<210> 82
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 82

Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro
1 5 10 15

<210> 83
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 83

Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu
1 5 10 15

<210> 84
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 84

Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met
1 5 10 15

<210> 85
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 85

Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln
1 5 10 15

<210> 86
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 86

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly
1 5 10 15

<210> 87
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 87

Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys
1 5 10 15

<210> 88
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 88

Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu
1 5 10 15

<210> 89
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 89

Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met
1 5 10 15

<210> 90
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 90

Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr
1 5 10 15

<210> 91
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 91

Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr
1 5 10 15

<210> 92
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Gag Peptide

<400> 92

Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln
1 5 10 15

<210> 93
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 93

Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe
1 5 10 15

<210> 94
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 94

Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg
1 5 10 15

<210> 95
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 95

Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys
1 5 10 15

<210> 96
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 96

Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys
1 5 10 15

<210> 97

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 97

Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Glu Gly
1 5 10 15

<210> 98
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 98

Thr Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Lys
1 5 10 15

<210> 99
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 99

Phe Asn Cys Gly Lys Glu Gly His Ile Ala Lys Asn Cys Arg Ala
1 5 10 15

<210> 100
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 100

Lys Glu Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys
1 5 10 15

<210> 101
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 101

Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys
1 5 10 15

<210> 102
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 102

Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu
1 5 10 15

<210> 103
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 103

Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met
1 5 10 15

<210> 104
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 104

Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr
1 5 10 15

<210> 105
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 105

Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala
1 5 10 15

<210> 106
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 106

His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly

1 5 10 15

<210> 107
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 107

Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro
1 5 10 15

<210> 108
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 108

Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly
1 5 10 15

<210> 109
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 109

Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn
1 5 10 15

<210> 110
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 110

Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser
1 5 10 15

<210> 111
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 111

His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro
1 5 10 15

<210> 112
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 112

Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro
1 5 10 15

<210> 113
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 113

Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe
1 5 10 15

<210> 114
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 114

Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu
1 5 10 15

<210> 115
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 115

Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Thr Thr Thr
1 5 10 15

<210> 116
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 116

Glu Ser Phe Arg Phe Gly Glu Glu Thr Thr Thr Pro Ser Gln Lys
1 5 10 15

<210> 117
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 117

Phe Gly Glu Glu Thr Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile
1 5 10 15

<210> 118
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 118

Thr Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu
1 5 10 15

<210> 119
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 119

Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala
1 5 10 15

<210> 120
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 120

Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser
1 5 10 15

<210> 121
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 121

Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn
1 5 10 15

<210> 122
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 122

Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser
1 5 10 15

<210> 123
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Gag Peptide

<400> 123

Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln
1 5 10

<210> 124
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 124

Met Arg Val Lys Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg
1 5 10 15

<210> 125
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 125

Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Gly Gly Thr Leu
1 5 10 15

<210> 126
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Env Peptide

<400> 126

Asn Tyr Gln His Leu Trp Arg Gly Gly Thr Leu Leu Leu Gly Met
1 5 10 15

<210> 127
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 127

Leu Trp Arg Gly Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys
1 5 10 15

<210> 128
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 128

Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys Ser Ala Val Glu
1 5 10 15

<210> 129
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 129

Leu Gly Met Leu Met Ile Cys Ser Ala Val Glu Lys Leu Trp Val
1 5 10 15

<210> 130
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 130

Met Ile Cys Ser Ala Val Glu Lys Leu Trp Val Thr Val Tyr Tyr
1 5 10 15

<210> 131

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 131

Ala Val Glu Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Ala
1 5 10 15

<210> 132
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 132

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Ala Trp Lys Glu Ala
1 5 10 15

<210> 133
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 133

Val Tyr Tyr Gly Val Pro Ala Trp Lys Glu Ala Thr Thr Thr Leu
1 5 10 15

<210> 134
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 134

Val Pro Ala Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser
1 5 10 15

<210> 135
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 135

Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala
1 5 10 15

<210> 136
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 136

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu
1 5 10 15

<210> 137
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 137

Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val
1 5 10 15

<210> 138
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 138

Ala Lys Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His
1 5 10 15

<210> 139
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 139

Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro
1 5 10 15

<210> 140
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 140

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn

1 5 10 15

<210> 141
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 141

Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Ile
1 5 10 15

<210> 142
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 142

Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Ile Val Leu Glu Asn
1 5 10 15

<210> 143
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 143

Asp Pro Asn Pro Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn
1 5 10 15

<210> 144
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 144

Pro Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met
1 5 10 15

<210> 145
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 145

Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn
1 5 10 15

<210> 146
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 146

Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln
1 5 10 15

<210> 147
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 147

Phe Asn Met Trp Lys Asn Asn Met Val Glu Gln Met His Glu Asp
1 5 10 15

<210> 148
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 148

Lys Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu
1 5 10 15

<210> 149
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 149

Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Gln Ser
1 5 10 15

<210> 150
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 150

His Glu Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Glu Pro Cys
1 5 10 15

<210> 151
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 151

Ile Ser Leu Trp Asp Gln Ser Leu Glu Pro Cys Val Lys Leu Thr
1 5 10 15

<210> 152
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 152

Asp Gln Ser Leu Glu Pro Cys Val Lys Leu Thr Pro Leu Cys Val
1 5 10 15

<210> 153
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 153

Glu Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu His Cys
1 5 10 15

<210> 154
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 154

Lys Leu Thr Pro Leu Cys Val Thr Leu His Cys Thr Asn Leu Glu
1 5 10 15

<210> 155
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 155

Leu Cys Val Thr Leu His Cys Thr Asn Leu Glu Asn Ala Thr Asn
1 5 10 15

<210> 156
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 156

Leu His Cys Thr Asn Leu Glu Asn Ala Thr Asn Thr Thr Ser Ser
1 5 10 15

<210> 157
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 157

Asn Leu Glu Asn Ala Thr Asn Thr Thr Ser Ser Asn Trp Lys Glu
1 5 10 15

<210> 158
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 158

Ala Thr Asn Thr Thr Ser Ser Asn Trp Lys Glu Met Asn Arg Gly
1 5 10 15

<210> 159
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 159

Thr Ser Ser Asn Trp Lys Glu Met Asn Arg Gly Glu Ile Lys Asn
1 5 10 15

<210> 160
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Env Peptide

<400> 160

Trp Lys Glu Met Asn Arg Gly Glu Ile Lys Asn Cys Ser Phe Asn
1 5 10 15

<210> 161
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 161

Asn Arg Gly Glu Ile Lys Asn Cys Ser Phe Asn Val Thr Thr Ser
1 5 10 15

<210> 162
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 162

Ile Lys Asn Cys Ser Phe Asn Val Thr Thr Ser Ile Gly Asn Lys
1 5 10 15

<210> 163
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 163

Ser Phe Asn Val Thr Thr Ser Ile Gly Asn Lys Met Gln Lys Glu
1 5 10 15

<210> 164
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 164

Thr Thr Ser Ile Gly Asn Lys Met Gln Lys Glu Tyr Ala Leu Phe
1 5 10 15

<210> 165

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 165

Gly Asn Lys Met Gln Lys Glu Tyr Ala Leu Phe Tyr Arg Leu Asp
1 5 10 15

<210> 166
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 166

Met Gln Lys Glu Tyr Ala Leu Phe Tyr Arg Leu Asp Val Val Pro
1 5 10 15

<210> 167
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 167

Tyr Ala Leu Phe Tyr Arg Leu Asp Val Val Pro Ile Asp Asn Asp
1 5 10 15

<210> 168
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 168

Tyr Arg Leu Asp Val Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
1 5 10 15

<210> 169
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 169

Val Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr Asn Leu Ile Asn
1 5 10 15

<210> 170
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 170

Asp Asn Asp Asn Thr Ser Tyr Asn Leu Ile Asn Cys Asn Thr Ser
1 5 10 15

<210> 171
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 171

Thr Ser Tyr Asn Leu Ile Asn Cys Asn Thr Ser Val Ile Thr Gln
1 5 10 15

<210> 172
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 172

Leu Ile Asn Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys
1 5 10 15

<210> 173
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 173

Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu
1 5 10 15

<210> 174
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 174

Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile

1 5 10 15

<210> 175
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 175

Cys Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala
1 5 10 15

<210> 176
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 176

Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe
1 5 10 15

<210> 177
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 177

Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys
1 5 10 15

<210> 178
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 178

Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys
1 5 10 15

<210> 179
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 179

Ala Gly Phe Ala Ile Leu Lys Cys Asn Asp Lys Lys Phe Asn Gly
1 5 10 15

<210> 180
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 180

Ile Leu Lys Cys Asn Asp Lys Lys Phe Asn Gly Ser Gly Pro Cys
1 5 10 15

<210> 181
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 181

Asn Asp Lys Lys Phe Asn Gly Ser Gly Pro Cys Ile Asn Val Ser
1 5 10 15

<210> 182
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 182

Phe Asn Gly Ser Gly Pro Cys Ile Asn Val Ser Thr Val Gln Cys
1 5 10 15

<210> 183
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 183

Gly Pro Cys Ile Asn Val Ser Thr Val Gln Cys Thr His Gly Ile
1 5 10 15

<210> 184
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 184

Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
1 5 10 15

<210> 185
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 185

Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu
1 5 10 15

<210> 186
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 186

His Gly Ile Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly
1 5 10 15

<210> 187
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 187

Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu
1 5 10 15

<210> 188
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 188

Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Gly Val Val
1 5 10 15

<210> 189
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 189

Leu Asn Gly Ser Leu Ala Glu Glu Gly Val Val Ile Arg Ser Glu
1 5 10 15

<210> 190
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 190

Leu Ala Glu Glu Gly Val Val Ile Arg Ser Glu Asn Phe Thr Asp
1 5 10 15

<210> 191
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 191

Gly Val Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Val Lys Thr
1 5 10 15

<210> 192
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 192

Arg Ser Glu Asn Phe Thr Asp Asn Val Lys Thr Ile Ile Val Gln
1 5 10 15

<210> 193
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 193

Phe Thr Asp Asn Val Lys Thr Ile Ile Val Gln Leu Lys Glu Ser
1 5 10 15

<210> 194
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Env Peptide

<400> 194

Val Lys Thr Ile Ile Val Gln Leu Lys Glu Ser Val Glu Ile Asn
1 5 10 15

<210> 195
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 195

Ile Val Gln Leu Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro
1 5 10 15

<210> 196
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 196

Lys Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
1 5 10 15

<210> 197
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 197

Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile
1 5 10 15

<210> 198
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 198

Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Pro Ile Gly Pro
1 5 10 15

<210> 199

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 199

Asn Asn Thr Arg Lys Ser Ile Pro Ile Gly Pro Gly Lys Ala Phe
1 5 10 15

<210> 200
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 200

Lys Ser Ile Pro Ile Gly Pro Gly Lys Ala Phe Tyr Ala Thr Gly
1 5 10 15

<210> 201
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 201

Ile Gly Pro Gly Lys Ala Phe Tyr Ala Thr Gly Asp Ile Ile Gly
1 5 10 15

<210> 202
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 202

Lys Ala Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln
1 5 10 15

<210> 203
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 203

Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn
1 5 10 15

<210> 204
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 204

Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Glu
1 5 10 15

<210> 205
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 205

Ile Arg Gln Ala His Cys Asn Ile Ser Gly Glu Lys Trp Asn Asn
1 5 10 15

<210> 206
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 206

His Cys Asn Ile Ser Gly Glu Lys Trp Asn Asn Thr Leu Lys Gln
1 5 10 15

<210> 207
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 207

Ser Gly Glu Lys Trp Asn Asn Thr Leu Lys Gln Ile Val Thr Lys
1 5 10 15

<210> 208
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 208

Trp Asn Asn Thr Leu Lys Gln Ile Val Thr Lys Leu Gln Ala Gln

1 5 10 15

<210> 209
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 209

Leu Lys Gln Ile Val Thr Lys Leu Gln Ala Gln Phe Glu Asn Lys
1 5 10 15

<210> 210
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 210

Val Thr Lys Leu Gln Ala Gln Phe Glu Asn Lys Thr Ile Val Phe
1 5 10 15

<210> 211
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 211

Leu Gln Ala Gln Phe Glu Asn Lys Thr Ile Val Phe Lys Gln Ser
1 5 10 15

<210> 212
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 212

Phe Glu Asn Lys Thr Ile Val Phe Lys Gln Ser Ser Gly Gly Asp
1 5 10 15

<210> 213
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 213

Thr Ile Val Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val
1 5 10 15

<210> 214
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 214

Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Met His Ser Phe
1 5 10 15

<210> 215
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 215

Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly
1 5 10 15

<210> 216
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 216

Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
1 5 10 15

<210> 217
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 217

His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr
1 5 10 15

<210> 218
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 218

Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn
1 5 10 15

<210> 219
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 219

Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn
1 5 10 15

<210> 220
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 220

Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Asn Asn Thr Ile Gly
1 5 10 15

<210> 221
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 221

Leu Phe Asn Ser Thr Trp Asn Asn Thr Ile Gly Pro Asn Asn Thr
1 5 10 15

<210> 222
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 222

Thr Trp Asn Asn Thr Ile Gly Pro Asn Asn Thr Asn Gly Thr Ile
1 5 10 15

<210> 223
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 223

Thr Ile Gly Pro Asn Asn Thr Asn Gly Thr Ile Thr Leu Pro Cys
1 5 10 15

<210> 224
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 224

Asn Asn Thr Asn Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
1 5 10 15

<210> 225
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 225

Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Arg
1 5 10 15

<210> 226
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 226

Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Arg Trp Gln Glu Val
1 5 10 15

<210> 227
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 227

Ile Lys Gln Ile Ile Asn Arg Trp Gln Glu Val Gly Lys Ala Met
1 5 10 15

<210> 228
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Env Peptide

<400> 228

Ile Asn Arg Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
1 5 10 15

<210> 229
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 229

Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly
1 5 10 15

<210> 230
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 230

Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys
1 5 10 15

<210> 231
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 231

Tyr Ala Pro Pro Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile
1 5 10 15

<210> 232
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 232

Ile Arg Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu
1 5 10 15

<210> 233

<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 233

Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp
1 5 10 15

<210> 234
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 234

Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Arg Glu
1 5 10 15

<210> 235
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 235

Gly Leu Leu Leu Thr Arg Asp Gly Gly Arg Glu Val Gly Asn Thr
1 5 10 15

<210> 236
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 236

Thr Arg Asp Gly Gly Arg Glu Val Gly Asn Thr Thr Glu Ile Phe
1 5 10 15

<210> 237
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 237

Gly Arg Glu Val Gly Asn Thr Thr Glu Ile Phe Arg Pro Gly Gly
1 5 10 15

<210> 238
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 238

Gly Asn Thr Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg
1 5 10 15

<210> 239
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 239

Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg
1 5 10 15

<210> 240
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 240

Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
1 5 10 15

<210> 241
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 241

Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val
1 5 10 15

<210> 242
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 242

Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu

1 5 10 15

<210> 243
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 243

Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val
1 5 10 15

<210> 244
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 244

Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys
1 5 10 15

<210> 245
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 245

Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg
1 5 10 15

<210> 246
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 246

Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg
1 5 10 15

<210> 247
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 247

Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala
1 5 10 15

<210> 248
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 248

Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Thr Leu Gly
1 5 10 15

<210> 249
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 249

Val Gln Arg Glu Lys Arg Ala Val Thr Leu Gly Ala Val Phe Leu
1 5 10 15

<210> 250
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 250

Lys Arg Ala Val Thr Leu Gly Ala Val Phe Leu Gly Phe Leu Gly
1 5 10 15

<210> 251
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 251

Thr Leu Gly Ala Val Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser
1 5 10 15

<210> 252
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 252

Val Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
1 5 10 15

<210> 253
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 253

Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Leu Thr
1 5 10 15

<210> 254
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 254

Ala Gly Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln
1 5 10 15

<210> 255
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 255

Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu
1 5 10 15

<210> 256
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 256

Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
1 5 10 15

<210> 257
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 257

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala
1 5 10 15

<210> 258
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 258

Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr Thr Thr Leu
1 5 10 15

<210> 259
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 259

Val Pro Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser
1 5 10 15

<210> 260
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 260

Glu Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val
1 5 10 15

<210> 261
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 261

Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro
1 5 10 15

<210> 262
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Env Peptide

<400> 262

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr
1 5 10 15

<210> 263
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 263

Gln Lys Glu Tyr Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile
1 5 10 15

<210> 264
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 264

Ala Leu Phe Tyr Lys Leu Asp Val Val Pro Ile Asp Asn Asp Asn
1 5 10 15

<210> 265
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Env Peptide

<400> 265

Gly Pro Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile
1 5 10 15

<210> 266
<211> 502
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 266

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp
1 5 10 15

Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys
20 25 30

```
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
        35                  40                  45

Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu
    50                  55                  60

Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
65                  70                  75                  80

Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Asp Val Lys Asp
                85                  90                  95

Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys
            100                 105                 110

Lys Lys Ala Gln Gln Ala Ala Ala Ala Ala Gly Thr Gly Asn Ser Ser
        115                 120                 125

Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met
    130                 135                 140

Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val
145                 150                 155                 160

Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala
                165                 170                 175

Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr
            180                 185                 190

Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn
        195                 200                 205

Glu Glu Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro
    210                 215                 220

Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly
225                 230                 235                 240

Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro
                245                 250                 255

Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
            260                 265                 270

Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg
        275                 280                 285

Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys
    290                 295                 300
```

```
Thr Leu Arg Ala Glu Gln Ala Ser Gln Asp Val Lys Asn Trp Met Thr
305                 310                 315                 320

Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu
                325                 330                 335

Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys
            340                 345                 350

Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala
        355                 360                 365

Met Ser Gln Val Thr Asn Pro Ala Asn Ile Met Met Gln Arg Gly Asn
    370                 375                 380

Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Glu
385                 390                 395                 400

Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp
                405                 410                 415

Arg Cys Gly Arg Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln
            420                 425                 430

Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys Gly Arg Pro Gly
        435                 440                 445

Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser
    450                 455                 460

Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro
465                 470                 475                 480

Ile Asp Lys Glu Leu Tyr Pro Leu Thr Ser Leu Arg Ser Leu Phe Gly
                485                 490                 495

Asn Asp Pro Ser Ser Gln
            500
```

<210> 267
<211> 487
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 267

```
Met Arg Val Lys Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Gly
1               5                   10                  15

Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys Ser Ala Val Glu Lys
            20                  25                  30

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr
```

```
        35                  40                  45

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
    50                  55                  60

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
65                  70                  75                  80

Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys
                85                  90                  95

Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp
            100                 105                 110

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu
        115                 120                 125

His Cys Thr Asn Leu Lys Asn Ala Thr Asn Thr Lys Ser Ser Asn Trp
    130                 135                 140

Lys Glu Met Asp Arg Gly Glu Ile Lys Asn Cys Ser Phe Lys Val Thr
145                 150                 155                 160

Thr Ser Ile Arg Asn Lys Met Gln Lys Glu Tyr Ala Leu Phe Tyr Lys
                165                 170                 175

Leu Asp Val Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr Lys Leu Ile
            180                 185                 190

Asn Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe
        195                 200                 205

Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu
    210                 215                 220

Lys Cys Asn Asp Lys Lys Phe Asn Gly Ser Gly Pro Cys Thr Asn Val
225                 230                 235                 240

Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln
                245                 250                 255

Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Gly Val Val Ile Arg Ser
            260                 265                 270

Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Lys Glu
        275                 280                 285

Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser
    290                 295                 300

Ile Thr Ile Gly Pro Gly Arg Ala Phe Tyr Ala Thr Gly Asp Ile Ile
```

305 310 315 320

Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Glu Lys Trp Asn
325 330 335

Asn Thr Leu Lys Gln Ile Val Thr Lys Leu Gln Ala Gln Phe Gly Asn
340 345 350

Lys Thr Ile Val Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val
355 360 365

Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr
370 375 380

Gln Leu Phe Asn Ser Thr Trp Asn Asn Thr Ile Gly Pro Asn Asn Thr
385 390 395 400

Asn Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Arg
405 410 415

Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln
420 425 430

Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly
435 440 445

Gly Lys Glu Ile Ser Asn Thr Thr Glu Ile Phe Arg Pro Gly Gly Gly
450 455 460

Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val
465 470 475 480

Lys Ile Glu Pro Leu Gly Val
485

<210> 268
<211> 8
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 268

Ser Ile Ile Asn Phe Glu Lys Leu
1 5

<210> 269
<211> 8
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 269

Lys Val Val Arg Phe Asp Lys Leu
1 5

<210> 270
<211> 8
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 270

Cys Phe Asp Val Phe Lys Glu Leu
1 5

<210> 271
<211> 24
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 271
ggggaattca tggagccagt agat 24

<210> 272
<211> 24
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 272
caagaattcc tattccttcg ggcc 24

<210> 273
<211> 20
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 273
cgagctgcaa gaactcttcc 20

<210> 274
<211> 20
<212> PRT
<213> Artificial

<220>
<223> PCR Primer

<400> 274

Ala Gly Gly Cys Cys Thr Thr Cys Cys Ala Thr Cys Thr Gly Thr Thr
1 5 10 15

Gly Cys Thr Gly
20

<210> 275
<211> 21

<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 275
gaagcatcca ggaagtcagc c 21

<210> 276
<211> 21
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 276
accttcttct tctattccgg g 21

<210> 277
<211> 30
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 277
tgacggggtc acccacactg tgcccatcta 30

<210> 278
<211> 30
<212> DNA
<213> Artificial

<220>
<223> PCR Primer

<400> 278
agtcatagtc cgcctagaag catttgcggt 30

<210> 279
<211> 14
<212> PRT
<213> Artificial

<220>
<223> synthetic peptide

<400> 279

Cys Leu Gly Gly Leu Leu Thr Met Val Ala Gly Ala Val Trp
1 5 10

<210> 280
<211> 9
<212> PRT
<213> Artificial

<220>
<223> EBNA4-derived peptide

<400> 280

Ile Val Thr Asp Phe Ser Val Ile Lys
1 5

<210> 281
<211> 10
<212> PRT
<213> Artificial

<220>
<223> EBNA4-derived peptide

<400> 281

Ala Val Phe Ser Arg Lys Ser Asp Ala Lys
1 5 10

<210> 282
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Lmp2-derived peptide

<400> 282

Cys Leu Gly Gly Leu Leu Thr Met Val
1 5

<210> 283
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Lmp2-derived peptide

<400> 283

Tyr Leu Gln Gln Asn Trp Trp Thr Leu
1 5

<210> 284
<211> 86
<212> PRT
<213> Human immunodeficiency virus

<400> 284

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Val Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr

50 55 60

His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

<210> 285
<211> 40
<212> PRT
<213> Human immunodeficiency virus

<400> 285

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser
1 5 10 15

Gln Thr His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg
20 25 30

Gly Asp Pro Thr Gly Pro Lys Glu
35 40

<210> 286
<211> 86
<212> PRT
<213> Artificial

<220>
<223> Tat Cys 22 mutant

<400> 286

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15

Gln Pro Lys Thr Ala Gly Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Val Cys Phe Ile Thr Lys Ala Leu Gly Ile Ser Tyr Gly
35 40 45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln Gly Ser Gln Thr
50 55 60

His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
65 70 75 80

Pro Thr Gly Pro Lys Glu
85

<210> 287
<211> 847
<212> PRT
<213> Human immunodeficiency virus type 1

<400> 287

Met Arg Val Lys Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Gly
1 5 10 15

Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys Ser Ala Val Glu Lys
20 25 30

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr
35 40 45

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
50 55 60

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
65 70 75 80

Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys
85 90 95

Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp
100 105 110

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu
115 120 125

His Cys Thr Asn Leu Lys Asn Ala Thr Asn Thr Lys Ser Ser Asn Trp
130 135 140

Lys Glu Met Asp Arg Gly Glu Ile Lys Asn Cys Ser Phe Lys Val Thr
145 150 155 160

Thr Ser Ile Arg Asn Lys Met Gln Lys Glu Tyr Ala Leu Phe Tyr Lys
165 170 175

Leu Asp Val Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr Lys Leu Ile
180 185 190

Asn Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe
195 200 205

Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu
210 215 220

Lys Cys Asn Asp Lys Lys Phe Asn Gly Ser Gly Pro Cys Thr Asn Val
225 230 235 240

Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln
245 250 255

Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Gly Val Val Ile Arg Ser

260 265 270

Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Lys Glu
275 280 285

Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser
290 295 300

Ile Thr Ile Gly Pro Gly Arg Ala Phe Tyr Ala Thr Gly Asp Ile Ile
305 310 315 320

Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Glu Lys Trp Asn
325 330 335

Asn Thr Leu Lys Gln Ile Val Thr Lys Leu Gln Ala Gln Phe Gly Asn
340 345 350

Lys Thr Ile Val Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val
355 360 365

Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr
370 375 380

Gln Leu Phe Asn Ser Thr Trp Asn Asn Thr Ile Gly Pro Asn Asn Thr
385 390 395 400

Asn Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Arg
405 410 415

Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln
420 425 430

Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly
435 440 445

Gly Lys Glu Ile Ser Asn Thr Thr Glu Ile Phe Arg Pro Gly Gly Gly
450 455 460

Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val
465 470 475 480

Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val
485 490 495

Val Gln Arg Glu Lys Arg Ala Val Thr Leu Gly Ala Met Phe Leu Gly
500 505 510

Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Arg Ser Leu Thr Leu
515 520 525

Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn

```
     530                 535                 540

Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr
545                 550                 555                 560

Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val Leu Ala Val Glu Arg
                565                 570                 575

Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys
            580                 585                 590

Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp Ser Asn Lys
        595                 600                 605

Ser Leu Asp Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Glu Arg
    610                 615                 620

Glu Ile Asp Asn Tyr Thr Asn Leu Ile Tyr Thr Leu Ile Glu Glu Ser
625                 630                 635                 640

Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys
                645                 650                 655

Trp Ala Ser Leu Trp Asn Trp Phe Asp Ile Ser Lys Trp Leu Trp Tyr
            660                 665                 670

Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu Val Gly Leu Arg Ile
        675                 680                 685

Val Phe Thr Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly Tyr Ser
    690                 695                 700

Pro Leu Ser Phe Gln Thr Arg Phe Pro Ala Pro Arg Gly Pro Asp Arg
705                 710                 715                 720

Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg Ser
                725                 730                 735

Ser Pro Leu Val His Gly Leu Leu Ala Leu Ile Trp Asp Asp Leu Arg
            740                 745                 750

Ser Leu Cys Leu Phe Ser Tyr His Arg Leu Arg Asp Leu Ile Leu Ile
        755                 760                 765

Ala Ala Arg Ile Val Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala Leu
    770                 775                 780

Lys Tyr Trp Gly Asn Leu Leu Gln Tyr Trp Ile Gln Glu Leu Lys Asn
785                 790                 795                 800

Ser Ala Val Ser Leu Phe Asp Ala Ile Ala Ile Ala Val Ala Glu Gly
```

805 810 815

Thr Asp Arg Ile Ile Glu Val Ala Gln Arg Ile Gly Arg Ala Phe Leu
820 825 830

His Ile Pro Arg Arg Ile Arg Gln Gly Phe Glu Arg Ala Leu Leu
835 840 845

<210> 288
<211> 502
<212> PRT
<213> Human immunodeficiency virus type 1

<220>
<221> linker
<222> (488)..(502)

<400> 288

Met Arg Val Lys Gly Ile Arg Lys Asn Tyr Gln His Leu Trp Arg Gly
1 5 10 15

Gly Thr Leu Leu Leu Gly Met Leu Met Ile Cys Ser Ala Val Glu Lys
20 25 30

Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr
35 40 45

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
50 55 60

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
65 70 75 80

Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys
85 90 95

Asn Asn Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp
100 105 110

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu
115 120 125

His Cys Thr Asn Leu Lys Asn Ala Thr Asn Thr Lys Ser Ser Asn Trp
130 135 140

Lys Glu Met Asp Arg Gly Glu Ile Lys Asn Cys Ser Phe Lys Val Thr
145 150 155 160

Thr Ser Ile Arg Asn Lys Met Gln Lys Glu Tyr Ala Leu Phe Tyr Lys
165 170 175

Leu Asp Val Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr Lys Leu Ile

```
            180                 185                 190

Asn Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser Phe
        195                 200                 205

Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala Ile Leu
    210                 215                 220

Lys Cys Asn Asp Lys Lys Phe Asn Gly Ser Gly Pro Cys Thr Asn Val
225                 230                 235                 240

Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln
                245                 250                 255

Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Gly Val Val Ile Arg Ser
            260                 265                 270

Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Lys Glu
        275                 280                 285

Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser
    290                 295                 300

Ile Thr Ile Gly Pro Gly Arg Ala Phe Tyr Ala Thr Gly Asp Ile Ile
305                 310                 315                 320

Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Gly Glu Lys Trp Asn
                325                 330                 335

Asn Thr Leu Lys Gln Ile Val Thr Lys Leu Gln Ala Gln Phe Gly Asn
            340                 345                 350

Lys Thr Ile Val Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val
        355                 360                 365

Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr
    370                 375                 380

Gln Leu Phe Asn Ser Thr Trp Asn Asn Thr Ile Gly Pro Asn Asn Thr
385                 390                 395                 400

Asn Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn Arg
                405                 410                 415

Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Arg Gly Gln
            420                 425                 430

Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly
        435                 440                 445

Gly Lys Glu Ile Ser Asn Thr Thr Glu Ile Phe Arg Pro Gly Gly Gly
```

```
     450                 455                 460

Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val
465                 470                 475                 480

Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val
                485                 490                 495

Val Gln Arg Glu Lys Arg
            500
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/011950

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61P35/00 A61P31/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 279 404 A (ISTITUTO SUPERIORE DI SANITA) 29 January 2003 (2003-01-29) cited in the application paragraph '0019! - paragraph '0037! claims; examples sequence 96	1-32
X	WO 98/17309 A (ERASMUS UNIVERSITY ROTTERDAM; VAN BAALEN, CAREL, A; OSTERHAUS, ALBERTU) 30 April 1998 (1998-04-30) page 4, line 11 - page 5, line 15 page 13, line 17 - page 14, line 17 claims	1,2,4, 20-25, 28,30,31

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2005	01/03/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rankin, R

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/27958 A (ISTITUTO SUPERIORE DI SANITA'; ENSOLI, BARBARA) 10 June 1999 (1999-06-10) page 1, line 1 - line 13 page 9, line 12 - line 30 page 17, line 4 - line 30 claims; examples	1-32
X	KIM D T ET AL: "Introduction of soluble proteins into the MHC class I pathway by conjugation to an HIV tat peptide" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 159, no. 4, 15 August 1997 (1997-08-15), pages 1666-1668, XP002083064 ISSN: 0022-1767 the whole document	1,2,4, 20-25, 27,28, 30,31
A	FANALES-BELASIO E ET AL: "Native HIV-1 Tat protein targets monocyte-derived dendritic cells and enhances their maturation, function, and antigen-specific T cell responses" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 168, 1 January 2002 (2002-01-01), pages 197-206, XP002226560 ISSN: 0022-1767 the whole document	1-32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/011950

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 31, 32 are directed to a method of treatment of the humanl body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/011950

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1279404 A	29-01-2003	EP 1279404 A1	29-01-2003
		BR 0211474 A	13-07-2004
		CA 2455227 A1	06-02-2003
		WO 03009867 A1	06-02-2003
		EP 1425035 A1	09-06-2004
		HU 0400660 A2	28-01-2005
WO 9817309 A	30-04-1998	US 6024965 A	15-02-2000
		AU 730887 B2	15-03-2001
		AU 4719597 A	15-05-1998
		CA 2268720 A1	30-04-1998
		EP 0930894 A1	28-07-1999
		WO 9817309 A1	30-04-1998
		JP 2001504812 T	10-04-2001
		KR 2000052649 A	25-08-2000
		NZ 335612 A	27-10-2000
		US 6319666 B1	20-11-2001
		US 5942401 A	24-08-1999
WO 9927958 A	10-06-1999	IT RM970743 A1	01-06-1999
		AU 762893 B2	10-07-2003
		AU 2412699 A	16-06-1999
		BR 9814725 A	03-10-2000
		CA 2311647 A1	10-06-1999
		CN 1283121 T	07-02-2001
		EA 4330 B1	29-04-2004
		WO 9927958 A2	10-06-1999
		EP 1035865 A2	20-09-2000
		JP 2001524531 T	04-12-2001
		NZ 505373 A	25-07-2003
		OA 11419 A	21-04-2004
		PL 341818 A1	07-05-2001
		TR 200001553 T2	21-11-2000